



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> : C12N 15/62, 15/13, C07K 16/30, 16/46, A61K 47/48, C07K 19/00, 14/705, C12N 9/64, 5/10, 1/21, 5/20 // A01K 67/027, A01H 1/00</p>	A1	<p>(11) International Publication Number: <b>WO 97/42329</b></p> <p>(43) International Publication Date: 13 November 1997 (13.11.97)</p>
<p>(21) International Application Number: PCT/GB97/01165</p> <p>(22) International Filing Date: 29 April 1997 (29.04.97)</p> <p>(30) Priority Data: 9609405.7 4 May 1996 (04.05.96) GB 9703103.3 14 February 1997 (14.02.97) GB</p> <p>(71) Applicant (for all designated States except US): ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): COPLEY, Clive, Graham [GB/GB]; Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). EDGE, Michael, Derek [GB/GB]; Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). EMERY, Stephen, Charles [GB/GB]; Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).</p>		<p>(74) Agent: GILES, Allen, Frank; Zeneca Pharmaceuticals, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: MONOCLONAL ANTIBODY TO CEA, CONJUGATES COMPRISING SAID ANTIBODY, AND THEIR THERAPEUTIC USE IN AN ADEPT SYSTEM</p> <p>(57) Abstract</p> <p>The invention relates to an anti-CEA monoclonal antibody (named "806.077 antibody") of murine origin and useful for the diagnosis and therapy of cancer. The antibody complementarity determining regions (CDRs) have the following sequences: heavy chain; CDR1 DNYMH, CDR2 WIDPENGDT EYAPKFRG, CDR3 LIYAGYLAMD Y; and light chain CDR1 SASSSVTYMH, CDR2 STSNLAS, CDR3 QQRSTYPLT. Humanised antibodies are described. The antibody is preferably in the form of a conjugate with either an enzyme suitable for use in an ADEPT system, especially a carboxypeptidase, or with a co-stimulatory molecule such as the extracellular domain of human B7.1.</p>		

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MONOCLONAL ANTIBODY TO CEA, CONJUGATES COMPRISING SAID ANTIBODY, AND  
THEIR THERAPEUTIC USE IN AN ADEPT SYSTEM

The present invention relates to a novel anti-CEA monoclonal antibody (named  
"806.077 antibody" or "806.077 Ab" herein) useful for the diagnosis and therapy of cancer.

5 It is established that the transformation of normal tissue cells to tumour cells is  
associated with a change in structure on the cell surface. Altered cell surface structures can  
serve as antigens and the tumour modified structures represent a type of so-called  
tumour-associated antigen (see for example *Altered Glycosylation in Tumour Cells*, Eds.  
Reading, Hakamori and Marcus 1988, Arthur R. Liss publ.). Such antigens may be exploited  
10 for example by the generation of monospecific antibodies using hybridoma technology as is  
presently well established after being first described by Kohler and Milstein (*Nature*, 256,  
495-497, 1975).

One tumour-associated antigen is CEA (Carcinomembryonic Antigen) as first  
described by Gold and Freedman, *J Exp Med*, 121, 439, 1965. This antigen is present on the  
15 tumour cell surface and can also be demonstrated in blood serum.

The concept of using antibodies to target tumour associated antigens in the treatment  
of cancer has been appreciated for some time (Herlyn et al. (1980) *Cancer Research* 40, 717).  
Antibodies may be used to target various chemical and biological agents to the tumour and  
such conjugates have been particularly successful in forming the basis for many methods of  
20 both in vitro and in vivo diagnosis. The use of immunoconjugates in the therapy of cancer is  
also promising (Lord et al. (1985) *Trends in Biotechnology* 3, 175; Vitetta et al (1987) *Science*  
238, 1098). This approach is technically more demanding than diagnostic applications and  
requires that tumour associated antigens which are targetted in such immunotherapeutic  
approaches, are highly tumour specific and not expressed at significant levels in vital human  
25 tissues. Whilst not wishing to be bound by theoretical considerations, as well as the property  
of having specific tumour associated tissue distribution, for some applications it is desirable  
that the antibody remain at the cell surface after antigen binding rather than being quickly  
internalised. For example in ADEPT (antibody directed enzyme prodrug therapy, see US  
patents 4975278 and 5405990) it is believed to be preferred that the antibody remain at the  
30 cell surface to facilitate prodrug activation by antibody-enzyme conjugate.

Antibody conjugates also have application in tumour immunotherapy. The following  
few paragraphs set out the scientific background for this application. In order to respond to an

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immune stimulus, T-cells require two signals. One such signal is provided by recognition of MHC displayed peptides by the T-cell receptor (TCR). It has been demonstrated however that TCR stimulation alone results in T-cell unresponsiveness or anergy and a second or co-stimulatory signal is required to stimulate specific T-cell activation and proliferation

5 (reviewed by Schwartz R.H. *J.Exp.Med.*, 1996, 184, 1-8). Upon receiving both signals, the resulting cytotoxic T-cells mediate the immune response by killing the target cells. A number of potential co-stimulatory molecules have been identified (eg B7, ICAMs, LFA-1 and 3, CD40, CD70 and CD24, reviewed by Galea-Lauri J. *et al* *Cancer Gene Therapy*, 1996, 3, 202-213). The major co-stimulatory function appears to be provided by the related molecules  
10 B7.1 (also called CD80) and B7.2 (also called CD86) which can interact with two receptors, CD28 and CTLA-4 (Hellstrom K.E. *et al* *Immunol. Rev.*, 1995, 145, 123-145 and Lenschow D.J. *et al* *Ann.Rev.Immunol.*, 1996, 14, 233-258). B7.1 and B7.2 are expressed on antigen presenting cells (APC) such as dendritic cells whereas CD28 and CTLA-4 are present on T-cells. B7.2 appears to be constitutively expressed on the surface of APCs but after contact  
15 with a T-cell, expression of B7.1 is up-regulated. Analogously, CD28 is expressed on T-cells but after activation is down-regulated and replaced by CTLA-4 expression. The stimulation of CD28 and CTLA-4 by B7.1 and B7.2 represents a complex pattern of signalling which controls not only the activation of the T-cell, but the subsequent control of proliferation to modulate the immune-response (Greene J. *et al* *J.Biol.Chem.*, 1996, 271, 26762-26771).  
20 This phenomenon may explain the sometimes conflicting data reported by workers studying these co-stimulatory molecules.

In cancer, tumour infiltrating lymphocytes have been identified but the lack of immune-response to the tumour may be due to T-cell anergy. Tumour cells can display specific or selective antigens on their surface but lack B7.1/B7.2 allowing them to escape  
25 immune surveillance. Indeed, *in vivo* experiments have demonstrated that B7.1/B7.2 transfected tumour cells are less tumourigenic than untransfected cells from the same line and that the transfected cells are capable of inducing protective immunity against rechallenge with parental cells (Townsend S.E. and Allison J.P., 1993, *Science*, 259, 368-370). This demonstrates that once stimulated, the immune response can become B7.1/B7.2 independent.  
30 Hellstrom has proposed that expression of B7.1/B7.2 in tumour cells by gene therapy has the potential to stimulate a host response which can reduce or eliminate the disease. Gajewski (*J.Immunol.*, 1996, 156, 465-472) and Matulonis *et al* (*J.Immunol.*, 1996, 156, 1126-1131)



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have reported that B7.1 is superior to B7.2 in the activation of T-cells. The use of B7.1 in solution (as a fusion with antibody constant domains) is reported to provide only modest co-stimulation to T-cells receiving TCR stimulation via an independent source (Linsley P.S. *et al* J.Exp.Med., 1991, 173, 721-730).

5        There is a need for further and improved anti-CEA antibodies useful in cancer diagnosis and therapy.

The present invention is based on the discovery of a novel anti-CEA antibody termed 806.077 antibody herein.

According to one aspect of the present invention there is provided an anti-CEA  
10 antibody comprising complementarity determining regions (CDRs) in which the CDRs have the following sequences:

a)        heavy chain

CDR1 DNYMH (SEQ ID NO: 29)

CDR2 WIDPENGDT E YAPKFRG (SEQ ID NO: 31)

15        CDR3 LIYAGYLAMD Y (SEQ ID NO: 32);

b)        light chain

CDR1 SASSSVTYMH (SEQ ID NO: 26)

CDR2 STSNLAS (SEQ ID NO: 27)

CDR3 QQRSTYPLT (SEQ ID NO: 28).

20        The CDRs or complementarity determining regions are those sequences within the hypervariable loops of antibody variable domains which are believed to be critical in determining the specificity of the antigen-antibody interaction (Kabat, E.A., Lu, T.T., Reid-Miller, M., Perry, H.M. & Gottesman, K.S. (1987). Sequences of Proteins of Immunological Interest. 4th edition. Washington D.C.: United States Dept. of Health and Human Services;  
25 the reader is also referred to this reference for details of Kabat antibody residue numbering). CDRs as defined herein however include framework residues where these contribute to binding. For the 806.077 antibody the CDRs were determined by homology with the hypervariable sequences of other murine antibodies. In this specification the terms "VK" and "VH" mean variable regions of the light and heavy antibody chains respectively. Anatomy of  
30 the antibody molecule has been reviewed by Padlan (1994) in Molecular Immunology 31, 169-217.

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**The Light Chain CDRs are:**

VK CDR1 Kabat residues 24-34 inclusive, SASSSVTYMH (SEQ ID NO: 26);

VK CDR2 Kabat residues 50-56 inclusive, STSNLAS (SEQ ID NO: 27);

VK CDR3 Kabat residues 89-97 inclusive, QQRSTYPLT (SEQ ID NO: 28);

**5 The Heavy Chain CDRs are:**

VH CDR1 Kabat residues 31-35B inclusive, DNYMH (SEQ ID NO: 29);

preferred VH CDR1 Kabat residues are no. 27-35B inclusive, FNIKDNYMH (SEQ ID NO: 30);

VH CDR2 Kabat residues 50-65 inclusive, WIDPENGDT E YAPKFRG (SEQ ID NO: 31)

10 VH CDR3 Kabat residues 95-102 inclusive, LIYAGYLAMD Y (SEQ ID NO: 32); and

preferred VH CDR3 Kabat residues are no. 93-102 inclusive, HVL IYAGYLA MDY (SEQ ID NO: 33).

Preferably binding affinity for CEA antigen is at least  $10E-5M$ , more preferably binding affinity for CEA is at least  $10E-6M$ , more preferably binding affinity for CEA is at least  $10E-7M$ , more preferably binding affinity for CEA is at least  $10E-8M$ , more preferably binding affinity for CEA is at least  $10E-9M$ , more preferably binding affinity for CEA is at least  $10E-10M$  and especially binding affinity for CEA is at least  $10E-11M$ .

The term antibody as used herein generally means an immunoglobulin molecule (or fragment thereof or modified antibody construct such as scFv which retains specific CEA antigen binding). The CDRs are principally responsible for antigen binding, the non-CDR protein sequence is normally derived from an immunoglobulin but may be derived from immunoglobulin domain of a immunoglobulin super family member.

According to another aspect of the present invention there is provided a CEA antibody comprising the following, optionally humanised, structure:

**25 a heavy chain variable region sequence (SEQ ID NO: 11)**

```
EVQLQQSGAE LVRSGASVKL SCTASGFNIK DNYMHWVKQR 40
PEQGLEWIAW IDPENGDT EY APKFRGKATL TADSSSNTAY 80
LHLSSLTSED TAVYYCHVLI YAGYLAMDY W GQGTSAVAVSS 120
and;
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**30 a light chain variable region sequence (SEQ ID NO: 9):**

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DIELTQSPAI MSASPGEKVT ITCSASSSVT YMHWFQKPG 40
TSPKLWIYST SNLASGVPAR FSGSGSGTSY SLTISRMEAE 80
DAATYYCQQR STYPLTFGAG TKLELKRA 108;
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or any one of the following constructs thereof:

F(ab')<sub>2</sub>; F(ab'), Fab, Fv, single chain Fv & V-min.

F(ab')<sub>2</sub> fragment constructs are preferred. Any suitable antibody fragment which retains 806.077 antibody binding characteristics is contemplated. For example a recently described antibody fragment is "L-F(ab)<sub>2</sub>" as described by Zapata (1995) in Protein Engineering, 8, 1057-1062. Disulphide bonded Fvs are also contemplated. Optionally the antibody forms part of a conjugate as described below.

A preferred humanised antibody comprises at least one of the following sequences:

- a heavy chain variable region sequence which is VH1 (SEQ ID NO: 55);
- 10 a light chain variable region sequence which is VK4 (SEQ ID NO: 71);
- a human CH1 heavy chain IgG3 constant region;
- a human kappa light chain CL region; and
- a human IgG3 hinge region;
- optionally in the form of a F(ab')<sub>2</sub> fragment.

- 15 According to another aspect of the present invention there is provided a polynucleotide sequence capable of encoding for the heavy or light chain variable region of a CEA antibody of the invention. Preferably the heavy or light chain variable region is fused (optionally via some linking sequence) to a gene encoding a protein effector moiety (as part of a conjugate, see text below), preferably fusion is through the antibody heavy chain. Generally fusion can
- 20 be either at the N or C terminus of the antibody chain. For B7 conjugates fusion at the N-terminus of the antibody chain is preferred.

- CPB has an N-terminal pro domain which is believed to assist correct folding of protein before the pro domain is removed to release active enzyme. If proCPB is fused at its C terminus to the N terminus of an antibody chain this allows removal of pro domain (e.g.
- 25 by trypsin treatment) from the N terminus of the fusion construct. Alternatively if proCPB was attached to the C terminus of an antibody chain then the problem arises of having to remove the pro domain from the "middle" of the construct without destroying the fusion protein. The solution is to co-express the pro domain separately (in trans). This has the advantage, once the cell lines have been constructed, of not requiring trypsin activation of
- 30 expressed fusion protein to remove CPB pro domain. Constructs with proCPB fused at its C terminus to the N terminus of an antibody chain have the advantage of not requiring

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construction of co-expression cell lines which require high level expression of the pro domain along with high level expression of other proteins..

In this specification conservative amino acid analogues of specific amino acid sequences are contemplated which retain the binding properties of the CEA antibody of the invention but differ in sequence by one or more conservative amino acid substitutions, deletions or additions. However the specifically listed amino acid sequences are preferred. Typical conservative amino acid substitutions are tabulated below.

#### Conservative Substitutions

Original	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Lys; Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Leu; Val; Ile; Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

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In this specification nucleic acid variations (deletions, substitutions and additions) of specific nucleic acid sequences are contemplated which retain which the ability to hybridise under stringent conditions to the specific sequence in question. A hybridisation test is set out in Example 9 hereinafter. However specifically listed nucleic acid sequences are preferred. It is contemplated that peptide nucleic acid may be an acceptable equivalent of polynucleotide sequences, at least for purposes that do not require translation into protein (Wittung (1994) *Nature* 368, 561).

According to another aspect of the present invention there is provided an antibody or antibody fragment as herein described characterised in that it is humanised.

10 A humanised antibody, related fragment or antibody binding structure is a polypeptide composed largely of a structural framework of human derived immunoglobulin sequences supporting non human derived amino acid sequences in and around the antigen binding site (complementarity determining regions or CDRs; this technique is known as CDR grafting which often involves some framework changes too, see the Examples below). Appropriate  
15 methodology has been described for example in detail in WO 91/09967, EP 0328404 and Queen et al. *Proc Natl Acad Sci* 86,10029, Mountain and Adair (1989) *Biotechnology and Genetic Engineering Reviews* 10, 1 (1992) although alternative methods of humanisation are also contemplated such as antibody veneering of surface residues (EP 519596, Merck/NIH, Padlan et al). Preferred humanised 806.077 antibodies are any one of Examples 11-47 or 107-  
20 122. A preferred humanised heavy chain variable region is VH1 (see Examples). A preferred light chain variable region is VK4 optionally incorporating any of the additional changes described in Examples 107-109. A preferred human heavy chain constant region is IgG3.

Chimaeric humanised antibodies represent another aspect of the invention. Preparation of chimaeric humanised antibody fragments of antibody 806.077 antibody is described in  
25 Example 8 herein. Chimaeric antibodies are generally constructed by combining the variable region from one species with a constant region from another antibody from a different species.

The term "humanised" in relation to antibodies as used herein includes any method of humanisation such as for example CDR grafting or chimaeric antibody preparation or any hybrid thereof such as for example a CDR grafted heavy chain in combination with a  
30 chimaerised light chain (see Example 110 for a suitable embodiment).

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In particular, a rodent antibody on repeated in vivo administration in man either alone or as a conjugate will bring about an immune response in the recipient against the rodent antibody; the so-called HAMA response (Human Anti Mouse Antibody). The HAMA response may limit the effectiveness of the pharmaceutical if repeated dosing is required. The immunogenicity of the antibody may be reduced by chemical modification of the antibody with a hydrophilic polymer such as polyethylene glycol or by using the methods of genetic engineering to make the antibody binding structure more human like. For example, the gene sequences for the variable domains of the rodent antibody which bind CEA can be substituted for the variable domains of a human myeloma protein, thus producing a recombinant chimaeric antibody. These procedures are detailed in EP 194276, EP 0120694, EP 0125023, EP 0171496, EP 0173494 and WO 86/01533. Alternatively the gene sequences of the CDRs of the CEA binding rodent antibody may be isolated or synthesized and substituted for the corresponding sequence regions of a homologous human antibody gene, producing a human antibody with the specificity of the original rodent antibody. These procedures are described in EP 023940, WO 90/07861 and WO91/09967. Alternatively a large number of the surface residues of the variable domain of the rodent antibody may be changed to those residues normally found on a homologous human antibody, producing a rodent antibody which has a surface 'vener' of residues and which will therefore be recognized as self by the human body. This approach has been demonstrated by Padlan et.al. (1991) Mol. Immunol. 28, 489.

According to another aspect of the present invention there is provided a host cell transformed with a polynucleotide sequence or a transgenic non-human animal or transgenic plant developed from the host cell in which the polynucleotide sequence encodes at least the variable region of the heavy chain or light chain of a CEA antibody of the invention, optionally in the form of a conjugate as described herein.

According to another aspect of the present invention there is provided hybridoma 806.077 deposited as ECACC deposit no. 96022936 and variant cell lines thereof.

Hybridoma 806.077 antibody was deposited at the European Collection of Animal Cell Cultures (ECACC), PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom on 29th February 1996 under accession no. 96022936 in accordance with the Budapest Treaty.

According to another aspect of the present invention there is provided plasmid pNG3-Vkss-HuCk deposited as NCIMB deposit no.40798.

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Plasmid pNG3-Vkss-HuCh was deposited at The National Collections of Industrial and Marine Bacteria (NCIMB), 23 St Machar Drive, Aberdeen AB2 1RY, Scotland, United Kingdom on 11-April-1996 under deposit reference number NCIMB 40798 in accordance with the Budapest Treaty.

- 5 According to another aspect of the present invention there is provided plasmid pNG4-VHss-HuIgG2CH1' deposited as NCIMB deposit no. 40797.

Plasmid pNG4-VHss-HuIgG2CH1' was deposited at The National Collections of Industrial and Marine Bacteria (NCIMB), 23 St Machar Drive, Aberdeen AB2 1RY, Scotland, United Kingdom on 11-April-1996 under deposit reference number NCIMB 40797 in  
10 accordance with the Budapest Treaty.

According to another aspect of the present invention there is provided plasmid pNG3-Vkss-HuCh-NEO deposited as NCIMB deposit no. 40799.

Plasmid pNG3-Vkss-HuCh-NEO was deposited at The National Collections of Industrial and Marine Bacteria (NCIMB), 23 St Machar Drive, Aberdeen AB2 1RY, Scotland,  
15 United Kingdom on 11-April-1996 under deposit reference number NCIMB 40799 in accordance with the Budapest Treaty.

According to another aspect of the present invention there is provided a method of making at least a variable region of a heavy or light chain of an anti-CEA antibody as herein defined comprising:

- 20 a) transforming a host cell with a polynucleotide sequence which encodes at least the variable region of the heavy or light chain of the anti-CEA antibody and optionally developing the transformed host cell into a transgenic non-human mammal or transgenic plant;
- b) subjecting the host cell, transgenic non-human mammal or transgenic plant to  
25 conditions conducive to expression, and optionally secretion, of at least the variable region and optionally;
- c) at least partially purifying the variable region.

According to another aspect of the present invention there is provided a method of making an antibody or a conjugate as defined herein which comprises:

- 30 a) subjecting a host cell, a transgenic non-human mammal or a transgenic plant as defined herein, or the 806.077 hybridoma, to conditions conducive to expression, and optionally secretion, of the antibody or conjugate; and optionally

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- b) at least partially purifying the antibody or conjugate.

Preferably both heavy and light chain variable regions are expressed in the same cell and assembled thereby to form an anti-CEA antibody. Preferably the heavy or light chain variable region is fused (optionally via some linking sequence) to a gene encoding a protein effector moiety (as part of a conjugate, see text below), preferably fusion is through the antibody heavy chain. Generally fusion can be either at the N or C terminus of the antibody chain. For B7 conjugates fusion at the N-terminus of the antibody chain is preferred. CPB has an N-terminal pro domain which is believed to assist correct folding of protein before the pro domain is removed to release active enzyme. If proCPB is fused at its C terminus to the N terminus of an antibody chain this allows removal of pro domain (e.g. by trypsin treatment) from the N terminus of the fusion construct. Alternatively if proCPB was attached to the C terminus of an antibody chain then the problem arises of having to remove the pro domain from the "middle" of the construct without destroying the fusion protein. The solution is to co-express the pro domain separately (in trans). This has the advantage, once the cell lines have been constructed, of not requiring trypsin activation of expressed fusion protein to remove CPB pro domain. Constructs with proCPB fused at its C terminus to the N terminus of an antibody chain have the advantage of not requiring construction of co-expression cell lines which require high level expression of the pro domain along with high level expression of other proteins..

According to another aspect of the present invention there is provided a method of making monoclonal antibody 806.077 comprising:

- a) culturing hybridoma 806.077 antibody deposited as ECACC deposit no. 96022936 in medium under conditions conducive to expression of antibody therefrom and;
- b) obtaining antibody 806.077 antibody from the culture medium and optionally;
- 25 c) preparing a  $F(ab')_2$  fragment of antibody 806.077 antibody by enzymic digestion.

According to another aspect of the present invention there is provided a conjugate which comprises an effector moiety and an anti-CEA 806.077 antibody of the invention as herein described. An effector moiety is an entity having the effect of bestowing another activity (e.g. an enzyme, toxin or radioactive ligand) to the 806.077 antibody in forming the conjugate.

In one embodiment, preferably the effector moiety is an enzyme suitable for use in an ADEPT system. In International Patent Application WO 96/20011, published 4-Jul-96, we



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proposed a "reversed polarity" ADEPT system based on mutant human enzymes having the advantage of low immunogenicity compared with for example bacterial enzymes. A particular host enzyme was human pancreatic CPB (see for example, Example 15 [D253K]human CPB & 16 [D253R]human CPB therein) and prodrugs therefor (see 5 Examples 18 & 19 therein). The host enzyme is mutated to give a change in mode of interaction between enzyme and prodrug in terms of recognition of substrate compared with the native host enzyme. In our subsequent International Patent Application No PCT/GB96/01975 (published 6-Mar-97 as WO 97/07796) further work on mutant CPB enzyme/ prodrug combinations for ADEPT are described. Preferred enzymes suitable for 10 ADEPT are any one of CPG2 or a reversed polarity CPB enzyme, for example any one of [D253K]HCPB, [G251T,D253K]HCPB or [A248S,G251T,D253K]HCPB.

806.077 Antibody conjugates also have application in tumour immunotherapy. Accordingly in another preferred embodiment the conjugate effector moiety is a co-stimulatory molecule, preferably the co-stimulatory molecule is B7, more preferably human 15 B7.1 or B7.2 and especially human B7.1. Preferably the conjugate is in the form of a fusion protein, preferably in which the fusion protein is formed through linking a C-terminus of the co-stimulatory molecule to an N-terminus 806.077 antibody chain, preferably via the antibody chain heavy chain, preferably in which the 806.077 antibody lacks an Fc antibody region, more preferably a F(ab')<sub>2</sub> antibody fragment, more preferably the antibody is humanised or 20 human. An especially preferred conjugate is described in Example 104 below.

The use of antibody to target a co-stimulatory molecule to tumour cells is predicted to bestow the function of antigen presenting to the tumour cells such that T-cells receive specific TCR stimulation from the tumour cell itself and a co-stimulatory signal from the antibody targeted molecule. The use of human or humanised antibodies is preferred for the treatment 25 of human tumours because murine antibodies may evoke an immune reaction when used in man which might result in a reduction in effectiveness on repeat therapy. The use of a fusion protein combining a tumour antigen binding region linked to the extracellular portion of a co-stimulatory molecule is novel. Hayden et al (Tissue Antigens, 1996, 48, 242-254) have reported the use of a bi-specific antibody molecule combining an anti-tumour antigen binding 30 domain with an anti-CD28 binding domain. Whilst this molecule is capable of interacting with CD28 on T-cells, the signal it may deliver has the disadvantage of being qualitatively different from that provided by the natural CD28 ligands, for example the affinity of binding

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is greater than that between B7.1 and CD28. The cross-species homology between B7.1, B7.2 and CD28, CTLA-4 indicates evolutionary conservation of binding region sequences.

Consequently it is believed that, for example B7.1 from man can interact with CD28 from mouse and may impart a similar co-stimulatory signal. For treatment of human disease a human or humanised protein is preferable. However, the use of a human or humanised protein in animal models could produce similar effects to that anticipated in man and such animal models should provide relevant data as to the efficacy of a human/humanised antibody fusion protein with human B7.1/B7.2 in the treatment of human disease.

Conjugation of the effector moiety and antibody may be by any suitable method such as for example chemical linkage via heterobifunctional linkers or recombinant gene fusion techniques. In general fusion proteins are preferred conjugates, particularly for conjugates with HCPB or B7.

Preferred conjugates are those in which the effector moiety is selected from any one of the following:

- 15 a) an enzyme suitable for use in an ADEPT system;
- b) CPG2;
- c) [G251T,D253K]HCPB;
- d) [A248S,G251T,D253K]HCPB;
- e) a co-stimulatory molecule;
- 20 f) extracellular domain of B7;
- g) extracellular domain of human B7.1; and
- h) extracellular domain of human B7.2;

optionally in the form of a fusion protein.

It will be appreciated that the conjugate of the present invention does not necessarily consist of one effector molecule and one antibody molecule. For example the conjugate may comprise more than one effector molecule per antibody molecule. In general,  $F(ab')_2$  antibody conjugates which are fusions between the antibody and an enzyme or an extracellular domain of B7 will have 2 moles of enzyme or B7 per mole of antibody.

Especially preferred conjugates are a fusion protein selected from any one of the following conjugates, (sequences being listed in N terminus to C terminus direction):

- a) a humanised 806.077  $F(ab')_2$  - {[A248S,G251T,D253K]HCPB}<sub>2</sub> fusion comprising:

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- an antibody Fd' chain of structure VH1(SEQ ID NO: 55)/CH1 constant region from IgG3/hinge region from IgG3;  
 the Fd' chain being fused via its C terminus to the N terminus of [A248S,G251T,D253K]HCPB; and
- 5 an antibody light chain of formula VK4(SEQ ID NO: 71)/CL region from kappa light chain;
- b) {[A248S,G251T,D253K]HCPB}<sub>2</sub>-humanised 806.077 F(ab')<sub>2</sub> fusion comprising: [A248S,G251T,D253K]HCPB;  
 the HCPB being fused at its C terminus, via a (GGGS)<sub>3</sub> linker, to the N terminus of an antibody Fd' chain of structure VH1(SEQ ID NO: 55)/CH1 constant region from IgG3/hinge
- 10 region from IgG3; and
- an antibody light chain of formula VK4(SEQ ID NO: 71)/CL region from kappa light chain; and
- c) a (human B7.1 extracellular domain)<sub>2</sub> - humanised 806.077 F(ab')<sub>2</sub> fusion comprising: human B7.1 extracellular domain;
- 15 the B7.1 being fused at its C terminus to the N terminus of an antibody Fd' chain of structure VH1(SEQ ID NO: 55)/CH1 constant region from IgG3/hinge region from IgG3; and
- an antibody light chain of structure VK4(SEQ ID NO: 71)/CL region from kappa light chain.

In this specification the antibody hinge region in relation to conjugates is defined according to the principles set out by Padlan (1994) in Molecular Immunology 31, 169-217;

20 see Table 2 therein in particular. In these especially preferred conjugates there are 2 moles of enzyme or B7.1 per mole of F(ab')<sub>2</sub>. The forward slash ("/") is merely a separator to indicate discrete structural elements joined by peptide bonds that make up parts of the conjugate.

The VH1 and/or VK4 variable region humanised sequences have the advantage of preserving good binding properties with minimal additional changes required to the human

25 framework. The IgG3 hinge region has the advantage of giving good F(ab')<sub>2</sub> production levels and homogeneity of product.

In another preferred embodiment relating to the especially preferred conjugates defined above, fusion is effected through the antibody light chain. In yet another preferred embodiment relating to the especially preferred conjugates defined above, the CH1 constant

30 region from IgG3/hinge region from IgG3 structural element may be replaced by the corresponding IgG2 element.

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When the effector molecule is a toxin, this toxin moiety generally comprises a component which possesses cytotoxic properties and hence is capable of killing cells following internalisation.

The toxin moiety and the anti-CEA antibody may be coupled directly to one another, 5 or they may be coupled indirectly. The toxin moiety and the anti-CEA antibody are, in general, coupled such that the geometry of the conjugate permits the anti-CEA antibody to bind to its target cell. Advantageously, the toxin moiety and the anti-CEA antibody are coupled such that the conjugate is extracellularly stable, and intracellularly unstable so that the toxin moiety and the anti-CEA antibody remain coupled outside the target cell, but 10 following internalisation, the toxin moiety is released. Thus, advantageously the conjugate has an intracellularly cleavable/extracellularly stable site.

Examples of conjugates in which the toxin moiety is directly coupled to the target cell binding moiety include those in which the toxin moiety and the anti-CEA antibody are coupled by a disulphide bridge formed between a thiol group on the toxin moiety and a thiol 15 group on the anti-CEA antibody. Details of the preparation and properties of immunotoxins and other conjugates are given in European patent application EP 528 527 (publication no.) the contents of which is incorporated herein by reference thereto.

According to another aspect of the present invention there is provided a polynucleotide sequence capable of encoding a polypeptide of an antibody or a conjugate as defined in any 20 preceding claim. The term "capable of encoding" is intended to encompass polynucleotide sequences taking into account degeneracy in the genetic code in that some amino acids are encoded by more than one codon.

According to another aspect of the present invention there is provided a vector comprising a polynucleotide as defined above.

25 According to another aspect of the present invention there is provided a host cell transformed with a polynucleotide sequence as defined above or a transgenic non-human animal or transgenic plant developed from the host cell.

According to another aspect of the present invention there is provided a pharmaceutical composition comprising a conjugate of the invention described herein in 30 association with a pharmaceutically-acceptable diluent or carrier, optionally in a form suitable for intravenous administration.

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According to another aspect of the present invention there is provided a conjugate as described herein for use as a medicament.

According to another aspect of the present invention there is provided use of a conjugate as described herein for preparation of a medicament for treatment of neoplastic  
5 disease.

It will be appreciated that the dose and dosage regimen will depend upon the particular effector moiety employed, the population of the target cell and the patient's history. The dose of the conjugate administered will typically be in the range 0.1 to 1mg/kg of patient weight.

The conjugates of the present invention will normally be administered in the form of a  
10 pharmaceutical composition. Thus according to the present invention there is also provided a pharmaceutical composition which comprises a conjugate (as defined herein) in association with a pharmaceutically-acceptable diluent or carrier. An example of such a formulation is given herein in Example 10.

Pharmaceutical compositions of the present invention may be formulated in a variety  
15 of dosage forms. Generally, the conjugates of the present invention will be administered parenterally, preferably intravenously. A particular parenteral pharmaceutical composition is one which is formulated in a unit dosage form which is suitable for administration by injection. Thus, particularly suitable compositions comprise a solution, emulsion or suspension of the immunotoxin in association with a pharmaceutically acceptable parenteral  
20 carrier or diluent. Suitable carriers or diluents include aqueous vehicles, for example water or saline, and non-aqueous vehicles, for example fixed oils or liposomes. The compositions may include agents which enhance the stability of the conjugate in the composition. For example, the composition may include a buffer. The concentration of the conjugate will vary, but in general, the conjugate will be formulated at concentrations of about 1 to 10 mg/ml.

25 According to another aspect of the present invention there is provided an expression vector coding for an anti-CEA antibody of the invention as herein defined.

According to another aspect of the present invention there is provided an expression vector encoding at least the variable region of a heavy or light chain of an anti-CEA antibody as herein defined.

30 According to another aspect of the present invention there is provided a host cell transformed with a vector as herein described which is compatible with expression therein.

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According to another aspect of the present invention there is provided a host cell transformed with a polynucleotide sequence as herein defined.

Mammalian cells (CHO, COS, myeloma) have been used as host for the co-expression of antibody H and L chain cDNAs and fragments thereof to produce antibody with the specified binding activity (Bebbington, C., 1991, Methods, vol 2, p136-145, and Adair, J., 1992, Immunological Reviews, vol 130). For expression of constructs leading to direct expression of active CPB, COS or CHO cell expression systems are preferred. The cDNAs can be introduced on plasmids and allowed to integrate into chromosomal DNA especially for CHO cells or allowed to replicate to very high copy number especially in COS cells. The plasmids generally require a selectable marker for maintenance in transfected hosts, an efficient eukaryotic promoter to allow a high level of transcription from the cDNAs, convenient restriction enzyme sites for cloning and polyadenylation and transcription termination signals for message stability. Several such vectors have been described in the literature (Bebbington, C. et al, 1992, Bio/Technology, vol 10, p169-175, and Wright, A., 1991, Methods, vol 2, p125-135) and there are commercially available vectors, (such as pRc/CMV, Invitrogen Corp.) which are suitable.

The expression of a range of antibody fragments in E.coli is well documented (reviewed by Pluckthun, A., Immunological Reviews, 1992, vol 130, p151-188 and Skerra, A., Current Opinion in Immunology, 1993, vol 5, p256-262). Intracellular expression of Fd and L chains has been described (Cabilly, S., 1989, Gene, vol 85, p553-557) but this may require in vitro refolding and re-association of the chains (Buchner, J and Rudolph, R., 1991, Bio/Technology, vol 9, p157-162) to produce binding activity. A more efficient route to obtaining active antibody fragments is through periplasmic secretion (Better, M. et al, 1988, Science, vol 240, p1041-1043). The H and L chain components of the antibody fragment are co-expressed from a single plasmid. Each antibody chain is provided with a bacterial leader peptide which directs it to the E.coli periplasm where the leader is cleaved and the free chains associate to produce soluble and active antibody fragments. This process is believed to mimic the natural process in eukaryotic cells where the expressed antibody chains pass into the lumen of the endoplasmic reticulum prior to association into whole antibodies. This process often results in the presence of binding activity in the culture supernatant.

Some expression systems involve transforming a host cell with a vector; such systems are well known such as for example in E. coli, yeast and mammalian hosts (see Methods in

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- Enzymology 185, Academic Press 1990). Other systems of expression are also contemplated such as for example transgenic non-human mammals in which the gene of interest, preferably cut out from a vector and preferably in association with a mammary promoter to direct expressed protein into the animal's milk, is introduced into the pronucleus of a mammalian
- 5 zygote (usually by microinjection into one of the two nuclei (usually the male nucleus) in the pronucleus) and thereafter implanted into a foster mother. A proportion of the animals produced by the foster mother will carry and express the introduced gene which has integrated into a chromosome. Usually the integrated gene is passed on to offspring by conventional breeding thus allowing ready expansion of stock. Preferably the protein of interest is simply
- 10 harvested from the milk of female transgenic animals. The reader is directed to the following publications: Simons et al. (1988), Bio/Technology 6:179-183; Wright et al. (1991) Bio/Technology 9:830-834; US 4,873,191 and; US 5,322,775. Manipulation of mouse embryos is described in Hogan et al., "Manipulating the Mouse Embryo; A Laboratory Manual", Cold Spring Harbor Laboratory 1986.
- 15 Transgenic plant technology is also contemplated such as for example described in the following publications: Swain W.F. (1991) TIBTECH 9: 107-109; Ma J.K.C. et al (1994) Eur. J. Immunology 24: 131-138; Hiatt A. et al (1992) FEBS Letters 307:71-75; Hein M.B. et al (1991) Biotechnology Progress 7: 455-461; Duering K. (1990) Plant Molecular Biology 15: 281-294.
- 20 If desired, host genes can be inactivated or modified using standard procedures as outlined briefly below and as described for example in "Gene Targeting; A Practical Approach", IRL Press 1993. The target gene or portion of it is preferably cloned into a vector with a selection marker (such as Neo) inserted into the gene to disrupt its function. The vector is linearised then transformed (usually by electroporation) into embryonic stem (ES) cells (eg
- 25 derived from a 129/Ola strain of mouse) and thereafter homologous recombination events take place in a proportion of the stem cells. The stem cells containing the gene disruption are expanded and injected into a blastocyst (such as for example from a C57BL/6J mouse) and implanted into a foster mother for development. Chimaeric offspring can be identified by coat colour markers. Chimeras are bred to ascertain the contribution of the ES cells to the germ
- 30 line by mating to mice with genetic markers which allow a distinction to be made between ES derived and host blastocyst derived gametes. Half of the ES cell derived gametes will carry the gene modification. Offspring are screened (eg by Southern blotting) to identify those with

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a gene disruption (about 50% of progeny). These selected offspring will be heterozygous and therefore can be bred with another heterozygote and homozygous offspring selected thereafter (about 25% of progeny). Transgenic animals with a gene knockout can be crossed with transgenic animals produced by known techniques such as microinjection of DNA into pronuclei, sphaeroplast fusion (Jakobovits *et al.* (1993) *Nature* 362:255-258) or lipid mediated transfection (Lamb *et al.* (1993) *Nature Genetics* 5 22-29) of ES cells to yield transgenic animals with an endogenous gene knockout and foreign gene replacement.

ES cells containing a targeted gene disruption can be further modified by transforming with the target gene sequence containing a specific alteration, which is preferably cloned into a vector and linearised prior to transformation. Following homologous recombination the altered gene is introduced into the genome. These embryonic stem cells can subsequently be used to create transgenics as described above.

The term "host cell" includes any procaryotic or eucaryotic cell suitable for expression technology such as for example bacteria, yeasts, plant cells and non-human mammalian zygotes, oocytes, blastocysts, embryonic stem cells and any other suitable cells for transgenic technology. If the context so permits the term "host cell" also includes a transgenic plant or non-human mammal developed from transformed non-human mammalian zygotes, oocytes, blastocysts, embryonic stem cells, plant cells and any other suitable cells for transgenic technology.

According to another aspect of the present invention there is provided a method of treatment of a human or animal in need of such treatment which comprises administration to a human or animal of a pharmaceutically effective amount of a conjugate as herein described.

According to another aspect of the present invention there is provided a method of targeting an effector moiety to cells displaying antigen CEA in a mammal in need of such targeting which comprises administration of a pharmaceutically effective amount of an conjugate of the invention as herein defined.

According to another aspect of the present invention there is provided the use of an antibody as hereinbefore described in a diagnostic method.

One diagnostic method is immunoassay. An immunoassay for *in vitro* testing based upon the novel antibody according to the invention may be designed in accordance with conventional immunological techniques in the art, utilising the antibody according to the invention in a labelled or unlabelled form and determining the complex formation of the



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antibody with CEA in the sample to be tested. In one case, the antibody may be labelled with a detectable label, such as radiolabel, a chemiluminescer, a fluorescer or an enzyme label.

Alternatively the antibody is detected via a complex formed with a labelled substance or by non-labelling techniques, such as biosensor methods eg based upon surface plasmon

5 resonance. The sample may, for example, be in the form of a body fluid, such as serum, or a tissue preparation (histochemical assay).

For in vivo diagnostic purposes, the antibody according to the invention is provided with a suitable externally detectable label, such as eg. a radiolabel or a heavy metal atom, and administered to a subject whereupon the possible localised accumulation of antibody in the  
10 body is determined.

For the in vitro diagnosis of cancer the anti-CEA antibody can be conjugated to either enzymes such as horse radish peroxidase and bacterial luciferase which can generate a signal which can be measured or to fluorescent markers or radioisotopes which can be detected and quantitated directly. In a standard immunoassay system such conjugates provide a means of  
15 measuring the presence or absence of CEA in body tissues and consequently provides a rapid and convenient test for the diagnosis of tumour disease. See general descriptions of the methodology involved in Enzyme Immunoassay, E.T. Maggio, CRC Press and US 3690 8334, US 3791 932, US 3817 837, US 3850 578, US 3853 987, US 3867 517, US 3901 654, US 3935 074, US 3984 533, US 3996 345 and US 4098 876.

20 For the in vivo diagnosis of cancer, the anti-CEA antibody can be conjugated to isotopes of elements such as yttrium, technetium or indium or heavy metal isotopes which can be detected by whole body imaging cameras (see Larson, S.M., 1987, Radiology, 165, 297-304).

For the therapy of cancer, preferred embodiments involve an anti-CEA antibody that  
25 can be conjugated to an effector moiety which can kill the cancer cells directly or especially via activation of a suitable prodrug in an ADEPT system. In ADEPT selective killing of tumour cells is achieved by conjugating the anti-CEA antibody to an enzyme which is capable of catalysing the conversion of a non-toxic dose of a prodrug into a potent toxic drug compound. Administration of the conjugate leads to localization of the enzyme activity at the  
30 tumour site. Subsequent administration of the prodrug leads to local production of the toxic drug and selective kill at the tumour site. This approach is described in WO 88/07378, US

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4,975,278, US 5,405,990 and WO89/10140. Antibody 806.077 may also be used conjugated to a co-stimulatory molecule for tumour immunotherapy as described above.

Selective cell killing of tumour cells can also be achieved by conjugation of the anti-CEA antibody either directly or by chemical derivatization with macrocycle chelators containing high energy radioisotopes such as  $^{90}\text{Y}$ ,  $^{131}\text{I}$  and  $^{111}\text{In}$ . The anti-CEA antibody serves to localize the isotope to the tumour and the radiation emitted by the isotope destroys the DNA of the surrounding cells and kills the tumour.

Selective killing of tumour cells can also be achieved by conjugation of the anti-CEA antibody to cytotoxic and cytostatic drugs such as methotrexate, chlorambucil, adriamycin, daunorubicin and vincristine. These drugs have been used in the clinic for many years and the therapy they provide is often limited by non specific toxicity. Conjugation of these drugs to the CEA antibody enables these drugs to localize at the tumour site and thus increasing the dose of drug that can be delivered to the tumour without incurring unacceptable side effects from the action of such drugs on other tissues such as the bone marrow or nervous system.

The effectiveness of the antibody is in many applications improved by reducing the size of the antibody binding structure and thereby improving the tissue penetration and other pharmacodynamic properties of the pharmaceutical composition. This can be achieved by removing the Fc region of the antibody molecule either enzymically or by genetic engineering methods to produce a recombinant Fab' or F(ab')<sub>2</sub> fragment.

Genetic engineering methods can also be used to further reduce the size of the anti-CEA antibody. The Fv which contain the CDRs can be engineered and expressed in isolation and chemically cross linked for instance by the use of disulphide bridges. Alternatively, both the light and heavy chain domains making up the Fv structure may be produced as a single polypeptide chain (SCFv) by fusing the Fv domains with a linker peptide sequence from the natural C-terminus of one domain to the N-terminus of the other domain (see PCT/US/87/02208 and US 4704692). Alternatively, a single Fv domain may be expressed in isolation forming a single domain antibody or dAb as described by Ward et al Nature(1989) 341, 544. Another type of anti-CEA antibody contemplated is a V-min construct as disclosed in International Patent Application WO 94/12625 (inventors Slater & Timms).

Abbreviations used herein include:

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ADEPT	antibody directed enzyme prodrug therapy
APC	antigen presenting cell
CDRs	complementarity determining regions
CEA	Carcinoma Embryonic Antigen
CL	constant domain of antibody light chain
CPB	carboxypeptidase B
CPG2	carboxypeptidase G2
DAB	substrate 3,3'-diaminobenzidine tetrahydrochloride
DEPC	diethylpyrocarbonate
DMEM	Dulbecco's modified Eagle's medium
ECACC	European Collection of Animal Cell Cultures
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
FCS	foetal calf serum
Fd	heavy chain of Fab, Fab' or F(ab') <sub>2</sub> optionally containing a hinge
HAMA	Human Anti Mouse Antibody
HCPB	human carboxypeptidase B, preferably pancreatic
hinge (of an IgG)	a short proline rich peptide which contains the cysteines that bridge the 2 heavy chains
HRPO	horse radish peroxidase
NCA	non-specific cross reacting antigen
NCIMB	National Collections of Industrial and Marine Bacteria
PBS	phosphate buffered saline
PCR	polymerase chain reaction
preproCPB	proCPB with an N-terminal leader sequence
proCPB	CPB with its N-terminal pro domain
SDS-PAGE	sodium dodecyl sulphate - polyacrylamide gel electrophoresis

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TBS	Tris-buffered Saline
VH	variable region of the heavy antibody chain
VK	variable region of the light antibody chain

The invention is illustrated by the following non-limiting Examples (supported by Reference Examples which follow the Examples) in which:

Figure 1 shows anti-tumour activity of 806.077 antibody-CPG2 conjugate in an ADEPT

5 model;

Figure 2 shows a plasmid map of pCF009;

Figure 3 shows BIAcore data showing antibody-B7.1 fusion protein binding to immobilised CTLA4-Ig in which the solid line represents test binding and the dotted line is a blank control; and unless otherwise stated;

10 DNA is recovered and purified by use of GENECLAN™ II kit (Strattech Scientific Ltd. or Bio 101 Inc.). The kit contains: 1) 6M sodium iodide; 2) a concentrated solution of sodium chloride, Tris and EDTA for making a sodium chloride/ethanol/water wash; 3) Glassmilk- a 1.5 ml vial containing 1.25 ml of a suspension of a specially formulated silica matrix in water. This is a technique for DNA purification based on the method of Vogelstein  
15 and Gillespie published in Proceedings of the National Academy of Sciences USA (1979) Vol 76, p 615. Briefly, the kit procedure is as follows. To 1 volume of gel slice is added 3 volumes of sodium iodide solution from the kit. The agarose is melted by heating the mix at 55°C for 10 min then Glassmilk (5-10ml) is added, mixed well and left to stand for 10 min at ambient temperature. The glassmilk is spun down and washed 3 times with NEW WASH  
20 (0.5ml) from the kit. The wash buffer is removed from the Glassmilk which is to dry in air. The DNA is eluted by incubating the dried Glassmilk with water (5-10ml) at 55°C for 5-10 min. The aqueous supernatant containing the eluted DNA is recovered by centrifugation. The elution step can be repeated and supernatants pooled;

Competent E. coli DH5α cells were obtained from Life Technologies Ltd (MAX  
25 efficiency DH5α competent cells);

Mini-preparations of double stranded plasmid DNA were made using the RPM™ DNA preparation kit from Bio101 Inc. (cat. No 2070-400) or a similar product - the kit contains alkaline lysis solution to liberate plasmid DNA from bacterial cells and glassmilk in

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a spinfilter to adsorb liberated DNA which is then eluted with sterile water or 10mM Tris-HCl, 1mM EDTA, pH 7.5;

Serum free medium is OPTIMEM™ I Reduced Serum Medium, GibcoBRL Cat. No. 31985;

5 LIPOFECTIN™ Reagent (GibcoBRL Cat. No. 18292-011) is a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-n,n,n-trimethylammonium chloride (DOTMA) and dioleoyl phosphatidylethanolamine (DOPE) in membrane filtered water. It binds spontaneously with DNA to form a lipid-DNA complex - see Felgner et al. in Proc. Natl. Acad. Sci. USA (1987) 84, 7431;

10 G418 (sulphate) is GENETICIN™, GibcoBRL Cat. No 11811, an aminoglycoside antibiotic related to gentamicin used as a selecting agent in molecular genetic experiments;

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase; and

General molecular biology procedures can be followed from any of the methods  
15 described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

#### Example 1

##### **Discovery and establishment of hybridoma cell line 806.077**

20 BALB/C mice, 8 to 10 weeks old, were immunised subcutaneously with a primary dose of CEA (10µg) in phosphate buffered saline solution (0.1ml) and Freund's Complete adjuvant (0.1ml). Two weeks later and again 2 weeks later the animals were boosted with further doses of CEA (10µg) in phosphate buffered saline (0.1ml) mixed with Freund's Incomplete adjuvant (0.1ml). Thirty two weeks later the animals were given a final  
25 intravenous immunisation of CEA (10µg) in phosphate buffered saline and sacrificed three days later. The spleens were removed and prepared and fused with NS0 cells (available from the European Collection of Animal Cell Cultures under the accession No. 85110503) by standard methods (Kohler and Milstein, Nature (1975) 256, 495). The resulting cells were distributed into 96-well culture dishes and incubated for 2 weeks. The supernatants from the  
30 resulting hybridomas were screened by EIA (enzyme immunoassay). From a total of 1,824 wells generated from 5 fusions, 102 wells were positive against native CEA. In fusion 806, seventeen wells were found to be positive. The cells contained in these wells were cloned by

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limiting dilution, and the resulting clones tested by EIA. Lines from 10/17 original wells cloned successfully. One line, designated 806.077, has been deposited with the European Collection of Animal Cell Cultures under Accession No. 96022936. The table below provides a summary of the antibody generation programme that led to discovery of the 806.077 antibody hybridoma.

Antigen	housing	rest weeks	number fusions	number Wells tested	number CEA* +ve by EIA	number finally selected
Untreated CEA	normal	8-20	28	13,920	99	0
desialated CEA	normal	8-12	14	5,568	12*	0
conjugated CEA	normal	10-12	8	3,168	1	0
Untreated CEA	isolator	>30	5	1,824	102	3

\* tested against untreated CEA, desialated immunisations produced lots of +ves when tested against the immunogen

10

### Example 2

#### **Preparation of 806.077 antibody from deposited hybridoma cell line ECACC No. 96022936**

##### **2.1 Preparation from serum containing medium**

15 A 1ml cryopreserved ampoule was removed from storage in liquid nitrogen and rapidly thawed in a 37°C water bath. The contents were aseptically transferred to a sterile 15ml centrifuge tube. The cells were resuspended by dropwise addition of 10ml of Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) foetal calf serum (FCS) accompanied by gentle mixing. The suspension was centrifuged at 50 x g for 10 min, the

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supernatant aseptically removed and the pellet resuspended in 5ml of DMEM, 10% FCS and 1% L-glutamine in a 95% air 5% carbon dioxide pre-gassed 25ml tissue culture flask. The flask was incubated at 36.5°C in the dark.

After 3 days the flask was sub-cultured by passing the contents of the entire flask into a larger 75ml flask diluting with DMEM, 10% FCS and 1% L-glutamine (final viable density =  $2-3 \times 10^5$  cells/ml). Further expansion to 162ml flasks was performed in a similar manner.

Culture supernatants for purification were prepared in 500ml roller cultures in 850ml roller bottles. Cultures were seeded at  $2 \times 10^5$  viable cells/ml in pre-gassed roller bottles, rotated at 3rpm and incubated at 36.5°C. Cultures were grown to maturity and harvested typically 500-800 hours after inoculation when the cell viability was below 10% and IgG concentration had reached a maximum.

## 2.2 Treatment of culture harvests

After harvest, roller bottle culture supernatants were clarified by centrifugation at 60 x g for 30 minutes. Sodium azide (0.02% w/v) was added as a preservative to the clarified supernatant which was stored at 4°C in the dark until purification.

## 2.3 Purification of 806.077 antibody

806.077 Antibody hybridoma supernatant (3l) was adjusted to pH 7.5 with dilute aqueous sodium hydroxide and filtered through a 0.45µm filter (Millipore MILLIDISK™). The filtered antibody supernatant was loaded onto an affinity column of Protein G (for example Protein G Fast Flow SEPHAROSE™, Pharmacia product code 17.0618.03; 5cm i.d x 6.5cm = 130ml;) equilibrated in phosphate buffered saline ("PBS"; 8mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5mM KH<sub>2</sub>PO<sub>4</sub>, 150mM NaCl, 2.5mM KCl, pH 7.3, for example as available in tablet form for reconstitution from Oxoid) at 4°C at a flow-rate of 4ml/min. The column was washed with PBS (260ml) at the same flow rate and the antibody eluted with 100mM sodium citrate pH 2.6, collecting fractions and monitoring the eluate by UV absorption (280nm). The UV absorbing fractions containing the antibody, were bulked, immediately adjusted to pH 7 and concentrated to about 2mg/ml by ultrafiltration using a 30 kDa cut-off membrane (e.g. Amicon YM30). Dialysis, using a 6-8 kDa porosity cut-off membrane (e.g. SPECTRAPOR™ 1) membrane, into 50mM tris-HCl pH 7.0 buffer yielded 110mg 806.077 antibody, >95% pure by SDS-PAGE.

Example 3**Selectivity of 806.077 antibody**

To assess selectivity, many human normal and tumour tissues have been screened for reactivity with the antibody 806.077, using sensitive three-stage indirect immunohistology on 5 acetone-fixed, frozen cryostat sections.

Immunohistology was carried out on sections of human tissue obtained either at resection surgery or at post mortem. To preserve optimal morphology and antigenicity, tissues were obtained as fresh as possible, cut into small pieces (about 0.5cm<sup>3</sup>) and flash frozen in liquid nitrogen prior to storage at -80°C. Sections of tissue (6µ) were cut on a 10 cryostat, mounted on polylysine coated slides (e.g. blue TECHMATE™ slides, Dako) and fixed in ice-cold acetone for 2 minutes before being wrapped in foil and stored at -80°C.

Slides were allowed to defrost at room temperature before being unwrapped from the foil immediately prior to use. Each section was outlined with a diamond marker, and to each section was added either 100µl 806.077 antibody diluted to 2 µg/ml in Tris-buffered Saline 15 (TBS), or 100µl CEA/NCA reactive control (A5B7 antibody) at 2µg/ml in TBS, or 100µl MOPC isotype control (Sigma Chemical Company, St. Louis, U.S.A., Cat. No. M 9269) at 2µg/ml in TBS, or relevant positive control such as LP34 (Dako). All subsequent incubations were carried out at room temperature for 30 minutes in a humidified chamber: all wash steps were in TBS with 2 changes. After incubation, the slides were washed and 100µl of second 20 antibody reagent, comprising 1/50 rabbit anti-mouse immunoglobulins conjugated to horse radish peroxidase (Dako Patts) and 1/5 normal human serum (Sigma) in TBS was added to each section.

The slides were again incubated and washed in TBS. A final detecting antibody, 100 µl swine anti-rabbit immunoglobulin conjugated to horse radish peroxidase (1/50 dilution 25 with 1/5 normal human serum in TBS), was added to each section, incubated and washed thoroughly. DAB substrate (3,3'-diaminobenzidine tetrahydrochloride) was prepared using 1 DAB tablet (Sigma) with hydrogen peroxide (17µl) in TBS (17ml), and added dropwise through a fast filter paper (e.g. Whatman Number 4). After 3 minutes incubation the excess DAB was tapped off the slides and the slides were washed in TBS. After counter staining 30 with haematoxylin (e.g. Mayer's Haematoxylin, Shandon) sections were dehydrated in alcohol and xylene, and mounted in non-aqueous synthetic mountant (e.g. E-Z mountant, Shandon) before examination under a microscope.



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The areas of antibody bonding were visualised by brown staining on the section. A scoring system was used to evaluate the degree of binding of 806.077 antibody to tissues:

- |               |  |
|---------------|--|
| +++ (strong)  | = antibody binding to >75% tumour cells                      |
| ++ (moderate) | = antibody binding to 50%- 75% tumour cells                  |
| 5 + (weak)    | = antibody binding to 25% - 50% tumour cells                 |
| +/- (minimal) | = non-focal antibody binding to a small area of tumour cells |
| -             | = no staining  |

Carcinoembryonic antigen (CEA) is a member of the immunoglobulin gene superfamily with one predicted variable-like domain region (N domain; 108 amino acids) and  
10 three sets of constant domain-like regions A1B1, A2B2 and A3B3; 92 amino acids for A domains and 86 amino acids for B domains (Hefta, 1992, Cancer Research 52:5647-5655. In addition, CEA possesses two signal peptides, one at the amino terminus and one at the carboxyl terminus. Both are removed during post-translational processing, the one at the carboxy terminus being replaced by a glycosylphosphatidylinositol (GPI) moiety.

15 A large number of CEA-related proteins with varying homology to CEA have been reported (Thompson, 1991, J. of Clinical Laboratory Analysis, 5: 344-366). These include non-specific cross reacting antigens, NCA 1 and 2. These related proteins are expressed on a range of normal tissues including granulocytes and normal lung epithelium. The majority of anti-CEA monoclonal antibodies generated so far, cross react with one of these related  
20 proteins and thus react with a range of normal tissues and often react strongly with either granulocytes or lung epithelium.

Anti-CEA antibody, 806.077 was identified as being CEA selective, exhibiting no cross reactivity to granulocytes and only minimal staining to 4/14 normal lung tissues tested.

806.077 antibody was initially screened for tumour and NCA selectivity as a tissue  
25 culture supernatant. The screens were carried out using the supernatant neat and diluted at 1:10 and demonstrated equivalent binding of the antibody to colon tumours when compared to A5B7, but much reduced binding to normal lung and spleen tissues when compared to the same antibody. The antibody was affinity purified (as described in Example 2) and the screens repeated and extended to include further tumours and tissue types.

30 The antibody was titrated against a panel of colo-rectal tumour sections and this screen demonstrated the optimum screening concentration of 806.077 antibody to be 2µg/ml. All subsequent screens were carried out using the antibody at this concentration.

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The results of these screens were as follows. The reactivity of 806.077 antibody was compared against A5B7 (also screened at 2µg/ml) against the following tumours/normal tissues:

**806.077 antibody Tumour reactivity:**

**5 Colon tumours (n = 17).**

Moderate to strong reactivity (++/+++ equivalent to A5B7) was seen to all 17 tumours tested .

**Breast tumours (n=6).**

Moderate/weak staining (+/+), 2/6 tumours; minimal staining(+/-), 2/6 tumours.

**NSCLC tumours (n=6).**

10 Strong staining (+++), 2/6 tumours; moderate staining (++), 1/6 and weak staining (+), 2/6 tumours.

**Gastric tumours (n=2).**

Strong staining (+++), 1/2 tumours; weak staining (+) 1/2 tumours.

**Ovary tumours (n=3) and prostate tumours (n=3).**

15 No staining was seen to any of these tumours.

In all cases, equivalent reactivity was seen with A5B7.

**Normal tissue reactivity:-**

**Lung (NCA reactivity) (n=14).**

Weak staining (+), 4/14 lung tissues; no staining (-) 10/14 tissues.

20 A5B7 bound moderately (++), 1/14 lung tissues; weakly (+), 10/14 tissues and minimally (+/-), 1/14 tissues.

**Spleen (granulocyte/NCA reactivity) (n=6).**

No staining was seen to any of the spleen tissues tested.

A5B7 bound moderately (++), 1/6 tissues and weakly (+), 5/6 tissues

**25 Post mortem normal tissues (n=13).**

Moderate/weak reactivity (++/+) was seen only to oesophagus, skin, colon and pancreas tissues (CEA expressing normal tissues). Similar binding was seen with A5B7. In addition to the positive tissues, colon, skin, oesophagus and pancreas, the negative tissues were: cerebellum, mid-brain, cerebrum, smooth muscle, liver, kidney, aorta, stomach, heart.

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**Example 4****Generation of 806.077 antibody F(ab') fragment**

Ficin (10mg) was suspended in a solution of 50mM cysteine (3ml; BDH 37218) and 50mM tris-HCl pH 7.0 and incubated at 37°C for 30 minutes. Excess cysteine was removed by size exclusion chromatography (Sephadex™ G-25 column, 1.5cm x 25cm; Pharmacia) in 50mM tris-HCl pH 7.0 buffer. The reduced ficin concentration was determined by monitoring UV absorbance at 280nm (assuming that a 1mg/ml solution has an absorbance reading of 2 in a 1cm cell) and was found to be 1.65mg/ml.

A solution of 806.077 antibody (100mg) in 50mM tris-HCl buffer pH 7.0 (50ml) and freshly reduced ficin (5mg; 3ml of the above solution) was digested at 37°C over 20 hours. The digest was then diluted with an equal volume of PBS and loaded onto a Protein G affinity column (Pharmacia SEPHAROSE™ Fast Flow, 5.0cm i.d x 6.5cm = 125ml; previously equilibrated with 50mM tris-HCl pH 7.0 buffer at 4°C), at a constant flow-rate of 3ml/min. The column was washed with 50mM sodium acetate pH 4.0 (250ml) to remove low M.W. fragments, followed by 50mM sodium citrate pH 2.8 to elute the F(ab')<sub>2</sub>, monitoring the UV absorbance of the eluate at 280nm. The F(ab')<sub>2</sub> containing eluate was adjusted to pH 7 and buffer exchanged into 100mM sodium phosphate/100mM sodium chloride/1mM EDTA, pH 7.2 by dialysis and concentrated to 8mg/ml by membrane filtration using a 10 kDa cut-off (e.g. Amicon™ YM10) assuming that a 1mg/ml solution has an absorbance reading at 280nm of 1.4 in a 1cm cell. A 65% yield of 42mg 95% pure F(ab')<sub>2</sub> was obtained.

**Example 5****Preparation of 806.077 antibody F(ab')<sub>2</sub> - carboxypeptidase G2 conjugate**

The linker for 806.077 antibody F(ab')<sub>2</sub> derivatisation was SATA (S-acetyl thioglycollic acid N-hydroxysuccinimide ester, Sigma, product code A 9043)

The linker for carboxypeptidase G2 (CPG2) derivatisation was SMPB [4-(p-maleimidophenyl) butyric acid N-hydroxysuccinimide ester, Sigma, product code M6139]

**5.1 F(ab')<sub>2</sub> derivatisation:**

To a solution of the F(ab')<sub>2</sub> fragment (40mg, prepared as described in Example 4) in 100mM phosphate/100mM NaCl/1mM EDTA pH 7.2 (buffer A; 5ml) was mixed with SATA (0.28mg) in DMSO (28μl). After 40 minutes at room temperature the resulting solution was applied to a desalting column (SEPHADEX™ G-25, 1.5cm i.d x 50cm = 100ml; equilibrated

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in buffer A at 4°C) at a flow-rate of 1.2ml/min. to remove excess reagents. The eluate was monitored by UV absorption at 280nm. The SATA derivatised F(ab')<sub>2</sub> was pooled and mixed with 10% v/v 500mM hydroxylamine HCl/500mM sodium phosphate/30mM EDTA pH 8.0 for 60minutes at room temperature to deacetylate the derivatised F(ab')<sub>2</sub>. The protein concentration was determined by UV absorption at 280nm assuming that a 1mg/ml solution has an absorbance reading of 1.4 in a 1cm cell. The solution was diluted to about 1mg/ml with buffer A. The linker loading was determined by Ellman's -SH assay and found to be 1.8-2.0 linkers / mole F(ab')<sub>2</sub>.

## 5.2 CPG2 derivatisation:

- 10 Large scale purification of CPG2 from Pseudomonas RS-16 was described in Sherwood et al. (1985), Eur. J. Biochem., 148, 447 - 453. Preparation of F(ab')<sub>2</sub> and IgG antibodies coupled to CPG enzyme may be effected by known means and has been described for example in PCT WO 89/10140. CPG may be obtained from Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom.
- 15 CPG2 may also be obtained by recombinant techniques. The nucleotide coding sequence for CPG2 has been published by Minton, N.P. et al., Gene, (1984) 31, 31-38. Expression of the coding sequence has been reported in E.coli (Chambers, S.P. et al., Appl. Microbiol, Biotechnol. (1988), 29, 572-578) and in Saccharomyces cerevisiae (Clarke, L. E. et al., J. Gen Microbiol, (1985) 131, 897-904). Total gene synthesis has been described by M. Edwards in 20 Am. Biotech. Lab (1987), 5, 38-44. Expression of heterologous proteins in E.coli has been reviewed by F.A.O. Marston in DNA Cloning Vol. III, Practical Approach Series, IRL Press (Editor D M Glover), 1987, 59-88. Expression of proteins in yeast has been reviewed in Methods in Enzymology Volume 194, Academic Press 1991, Edited by C. Guthrie and G R Fink.
- 25 CPG enzyme is available from Sigma Chemical Company, Fancy Road, Poole, Dorset, U.K. CPG enzyme was described in: Goldman, P. and Levy, C.C., PNAS USA, 58: 1299-1306 (1967) and in: Levy, C.C. and Goldman P., J. Biol. Chem., 242: 2933-2938 (1967). Carboxypeptidase G3 enzyme has been described in Yasuda, N. et al., Biosci. Biotech. Biochem., 56: 1536-1540 (1992). Carboxypeptidase G2 enzyme has been described 30 in European Patent 121 352.

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CPG2 (50mg; recombinant enzyme from *E. coli*) was dialysed into 100mM sodium phosphate/100mM sodium chloride pH 7.2 (=buffer B) and diluted to 8mg/ml, assuming that a 1mg/ml solution has an absorbance reading at 280nm of 0.6 in a 1cm cell.

SMPB(Sigma) was dissolved in DMSO at 10mg/ml. CPG2 (50mg in buffer B at 5 8mg/ml) was mixed with the SMPB solution (0.108ml; 1.08mg), and reacted at room temperature for 120 minutes. Excess reagents were removed on a desalting column (Sephadex G-25, 1.5cm i.d x 50cm = 100ml; equilibrated in buffer B at 4°C) at 1.2ml/min. Derivatised CPG2 was pooled and the concentration determined by UV A280nm, assuming that a 1mg/ml solution has an absorbance reading at 280nm of 0.6 in a 1cm cell. The solution 10 was diluted to a CPG2 concentration of about 1mg/ml. The linker loading was determined by a 'reverse' Ellman's assay, by adding a known amount of 2-mercaptoethanol to the maleimido-derivatised CPG2 and assaying unreacted -SH. A linker loading of 2.0-2.4 linkers / mole CPG2 was found.

### 5.3 Conjugation:

15 Equal weights of the deacetylated derivatised F(ab')<sub>2</sub> and derivatised CPG2 were mixed under nitrogen and the mixture (about 80ml, at a total protein concentration of about 1mg/ml) left at room temperature for 20h. The reaction was terminated by the addition of 10% v/v 100mM aqueous glycine. The crude conjugation mixture was buffer exchanged by dialysis into a low salt buffer (50mM sodium acetate pH 6.0) and applied to a dye-ligand 20 affinity column (where the dye binds to CPG2 e.g. ACL Mimetic Green 1, 2.5cm i.d x 10cm = 50ml) at 4°C equilibrated in the same buffer, to remove unreacted derivatised F(ab')<sub>2</sub>. The conjugate and derivatised CPG2 were eluted with 50mM acetate/ 500mM NaCl pH 6.0, at a flow rate of 2.0ml/min monitoring the elution by UV (A280nm).

The crude conjugate, still containing derivatised CPG2, was concentrated using a 10 25 kDa cut-off ultrafiltration device (e.g. Amicon YM10™) to about 12ml, at 5mg/ml total protein concentration and 10% v/v 10mM zinc sulphate (Sigma Z 0251) in water was added to replenish zinc lost to the CPG2 in the process. Further chromatography by size exclusion (e.g. SEPHACRYL S-300HR™ Pharmacia, 2.5cm i.d x 25cm = 500ml) at 4°C in 50mM sodium acetate / 150mM sodium chloride pH 6.0 at a flow-rate of 1ml/min., collecting 30 fractions and monitoring by UV A280nm, resulted in the fractionation of the conjugate and its separation from unreacted derivatised CPG2, as determined by SDS-PAGE of column fractions.

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The peak containing conjugate (with ratios of  $F(ab')_2$ :CPG2 of 1:2, 1:1 and 2:1) was pooled and concentrated by ultrafiltration to 1.3mg/ml, the protein concentration being determined by monitoring UV adsorbance at A280nm (assuming 1mg/ml has an absorbance of 1.0). Purity of the conjugate was determined by SDS PAGE and found to contain a total of 5 12mg conjugate with the composition 65% 1:1 ratio conjugate, 20% 1:2 or 2:1 ratio conjugate with < 5% free derivatised  $F(ab')_2$  and < 5% free derivatised CPG2.

#### Example 6

#### **Anti-tumour activity of 806.077 antibody $F(ab')_2$ -CPG2 conjugate in combination with 10 a prodrug.**

The anti-tumour activity of the 806.077 antibody  $F(ab')_2$ -CPG2 conjugate prepared as described in Example 5 was evaluated in combination with the prodrug N(4-[N,N-bis(2-chloroethyl)amino]-phenoxy carbonyl)-L-glutamic acid (called "PGP" in this example, is described in Example 1 in US 5,405,990 and Blakey et al., Br. J. Cancer 72, 1083-88, 1995)  
15 in a human colorectal tumour xenograft model.

Groups of 8-10 female athymic nude mice were injected s.c. with  $1 \times 10^7$  LoVo colorectal tumour cells (ECACC no 87060101). When the tumours were 4-5 mm in diameter either 806.077 antibody  $F(ab')_2$ -CPG2 conjugate (250 U CPG2 enzyme activity  $Kg^{-1}$ ) or phosphate buffered saline (170 mM NaCl, 3.4 mM KCl, 12 mM  $Na_2HPO_4$ , 1.8 mM  $KH_2PO_4$ ,  
20 pH 7.2) was injected intravenously (i.v). Seventy-two hours later PGP prodrug was injected i.p. (3 doses of 40 mg/Kg at 1 h intervals). The length of the tumours in two directions was then measured three times a week and the tumour volume calculated using the formula:

$$\text{Volume} = \pi/6 \times D^2 \times d$$

where D is the larger diameter and d is the smaller diameter of the tumour. Tumour volume  
25 was expressed relative to the tumour volume at the time of initiation of the prodrug arm of the therapy. The anti-tumour activity was compared with control groups given PBS instead of either conjugate or prodrug. Anti-tumour activity was expressed both as a growth delay defined as the time it takes treated tumours to increase their volume by 4-fold minus the time it takes control tumours to increase their volume 4-fold and as a T/C value defined as the  
30 volume of the treated tumour divided by the volume of the control tumour 14 days after prodrug administration. Statistical significance of the anti-tumour effects was judged using the analysis of variance (one-way) test.

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The anti-tumour activity of 806.077 antibody F(ab')<sub>2</sub>-CPG2 conjugate in combination with PGP prodrug are shown in Fig 1 and the anti-tumour data is summarised below.

**Anti-tumour activity of 806.077 antibody F(ab')<sub>2</sub>-CPG2 conjugate in combination with PGP prodrug in LoVo tumour xenografts.**

5

Conjugate	Dose (U/kg)	T/C (%)	Growth delay (days)	Significance (p)
806.077 F(ab') <sub>2</sub> -CPG2	250	16.5	14	<0.01
	500	4.7	22	<0.01

The results demonstrate that the 806.077 antibody F(ab')<sub>2</sub>-CPG2 conjugate in combination with the PGP prodrug produce tumour regressions and prolonged growth delays which were statistically significant compared with control groups.

10

#### Example 7

#### **Cloning and sequencing of the variable regions of 806.077 antibody heavy and light chain genes**

##### **7.1 Preparation of cytoplasmic RNA**

15 There are several procedures for the isolation of polyA<sup>+</sup> mRNA from eukaryotic cells (Sambrook J., Fritsch E.F., Maniatis T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Second Edition, 1989, Chapter 8, p3 herein referred to as "Maniatis"). In this particular case cytoplasmic RNA was prepared as described by Favoloro et al., Methods in Enzymology 65, 718-749, from a frozen hybridoma cell pellet containing  
20 1x10<sup>9</sup> cells which had been stored at -80°C.

The cells were resuspended in 5ml ice-cold lysis buffer (140mM NaCl, 1.5mM MgCl<sub>2</sub>, 10mM Tris-HCl pH 8.6 and 0.5% NP40 (a polyglycol ether nonionic detergent; Nonylphenoxy Polyethoxy Ethanol, Sigma Cat. No. 127087-87-0)) containing 400u of a ribonuclease inhibitor (RNAguard; Pharmacia Cat. No. 27-0815-01) and vortexed for 10s.  
25 This solution was overlayed on an equal volume of ice cold lysis buffer containing 24% (w/v) sucrose and 1% NP-40 and stored on ice for 5 min. The preparation was then centrifuged at 4000 rpm for 30 min at 4°C in a bench top centrifuge (Sorval RT6000B ) after which, the

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upper cytoplasmic phase was removed to an equal volume of 2 x PK buffer (200mM Tris (pH7.5), 25mM EDTA, 300mM NaCl and 2% (w/v) SDS). Proteinase K (Sigma, Cat No. P2308) was added to a final concentration of 200µg/ml and the mixture incubated at 37°C for 30 min.

5        The preparation was extracted with an equal volume of phenol/chloroform, the aqueous phase removed and 2.5 vol ethanol added and mixed. This solution was then stored at -20°C overnight. RNA was collected by centrifugation (4000 rpm, 30 min at 4°C in a bench top centrifuge, Sorval RT6000B), the supernatant decanted and the pellet dried in a vacuum dessicator after which it was dissolved in 250 µl diethylpyrocarbonate (DEPC)-  
10 treated water (prepared as described in Maniatis, referenced above). The RNA content was measured by spectrophotometry and the concentration calculated assuming an absorbance at 260nm of 1 = 40 µg/ml.

#### 7.2        Preparation of first strand variable region cDNA

A number of methods for the synthesis of cDNA are reviewed in Maniatis (Chapter  
15 8). The oligonucleotide primers used were mainly based on those proposed by Marks et al. J. Mol. Biol (1991) 222, 581-597. The cDNA in this case was prepared as described below. RNA (5mg) was mixed in a microcentrifuge tube with 10 µl 5x reverse transcriptase buffer [250mM Tris (pH8.3), 40mM MgCl<sub>2</sub> and 50mM DTT], 1µl forward primer (25 pM), 10µl 1.25mM dNTPs, 5µl 10 mM DTT, 0.5µl RNAGuard (Pharmacia) to which DEPC-treated  
20 H<sub>2</sub>O was added to obtain a volume of 50µl. The reaction mix was heated to 70°C for 10 min and then cooled slowly to 37°C, after which 100 u (0.5µl) M-MLV reverse transcriptase (Pharmacia Cat. No. 27-0925-01) were added and the reaction incubated at 37°C for 1 h. The forward primer used for the generation of the light chain cDNA was oligonucleotide CK2FOR (SEQ ID NO: 1) which is designed to hybridise to the CK constant region of  
25 murine kappa light chain genes. For the heavy chain cDNA the forward primer CG1FOR (SEQ ID NO: 2) was used which hybridises to the CH1 constant domain of murine IgG1.

#### 7.3        Amino acid sequencing

The heavy and light chains of the 806.077 antibodies were isolated by SDS-PAGE and Western blotting and submitted for N-terminal amino acid sequencing. The results  
30 showed that the N-terminus of the light chain was chemically blocked, however, sequence data was obtained for the first 34 N-terminal residues of the heavy chain (SEQ ID NO: 3).



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On the basis of this amino acid sequence a specific DNA back primer was designed for 806.077 heavy chain variable region PCR. This primer was called SP1back (SEQ ID NO: 7).

#### 7.4 Isolation of antibody gene fragments by PCR

Isolation of 806.077 heavy and light chain variable region genes was performed using the cDNA prepared as described above as template. General reaction conditions were as follows.

To 5µl of the cDNA reaction was added 5µl dNTPs (2.5mM), 5µl 10x Enzyme buffer (500mM KCl, 100mM Tris (pH8.3), 15mM MgCl<sub>2</sub> and 0.1% gelatin), 1µl of 25pM/µl back primer, 1µl of 25pM/µl forward primer, 0.5µl thermostable DNA polymerase and DEPC-treated water to obtain a volume of 50µl. The PCR conditions were set for 25 cycles at 94°C for 90s; 55°C for 60s; 72°C for 120s, ending the last cycle with a further 72°C for 10 min incubation.

Using the general reaction conditions, the forward primer used for the generation of the light chain cDNA was oligonucleotide CK2FOR (SEQ ID NO: 1) and the for the heavy chain cDNA oligonucleotide CG1FOR (SEQ ID NO: 2). A number of reactions using a variety of different back primers were performed for both the heavy and light chains to obtain desired specific PCR products.

In the case of the 806.077 light chain, on analysis a specific PCR product was obtained using the back primers VK1back (SEQ ID NO: 4) and VK4back (SEQ ID NO: 5). Similarly specific PCR products were obtained for the heavy chain using VH1back (SEQ ID NO: 6) and SP1back primers (SEQ ID NO: 7). Reaction products were analysed on a 2% agarose gel. Products of the expected size, were excised and the DNA purified.

#### 7.5 Cloning of the PCR products into Bluescript KS+ vector

For each antibody fragment, both the 5' region (back primer) oligonucleotide and the 3' region (forward primers) introduced a restriction site. The discrete PCR products were for both the VH and VK PCR reactions were therefore able to be cloned into the Bluescript vector KS+ (Stratagene Cloning Systems) via the appropriate enzyme restriction sites using standard DNA manipulation methods (e.g. PCR products VH1back/CG1For was cloned via PstI/HindIII and VK4back/CK2For via SacI/HindIII). DNA was prepared from the clones obtained and rigorous sequencing of at least 12 clones of each construct performed using automated fluorescent sequencing equipment (Applied Biosystems). The sequences were

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reviewed, compared and aligned using suitable computer software. Consensus sequences for both the VH and VK genes were obtained and subsequently translated to their corresponding amino acid sequence.

The DNA and amino acid sequences obtained for the 806.077 light chain variable (VK) region are described in SEQ ID NO: 8 and SEQ ID NO: 9 respectively. The DNA and amino acid sequences obtained for the 806.077 heavy chain variable (VH) region are described in SEQ ID NO: 10 and SEQ ID NO: 11 respectively. A clone containing the light chain was designated VK4, and a clone containing the heavy chain sequence was designated VH14A.

10

#### Example 8

#### **Construction of chimaeric light chain and heavy chain Fd genes**

The heavy and light chain genes which had been cloned into Bluescript (VK4 and VH14A in Example 7) were isolated by PCR using primers which allowed specific amplification of only the variable region the appropriate genes but also introduced new unique enzyme restriction sites. These restriction sites enabled the variable region gene fragments to be cloned in frame with DNA fragments coding for both the appropriate antibody signal sequences and human constant regions. The signal and constant region sequences for the light and heavy chain Fd had each been previously cloned into pNG3 and pNG4, derivatives of the pSG5 Eukaryotic plasmid expression vector.

The vector pNG3 was prepared as follows. Plasmid pSG5 (Stratagene, Cat. No. 216201) was digested with SalI and XbaI to remove the existing SV40 promoter and polylinker sequence. A new polylinker was introduced by use of oligonucleotides SEQ NOS: 34 and 35 which were hybridised and cloned into the SalI and XbaI cut pSG5 plasmid to give plasmid pNG1. The pNG1 plasmid was cut with BglII and HindIII and the BglII-HindIII CMV promoter fragment from pcDNA3 (Invitrogen, Cat. No. V790-20) cloned into this site to give plasmid pNG2. Finally, the polyA region from pSG5 was isolated by PCR as described in Example 7, section 7.4 but using oligonucleotide sequences SEQ ID NOS: 36 and 37 with plasmid pSG5. The PCR product was cut with XmaI and BamHI, purified by electrophoresis on a 2% agarose gel, isolated (e.g. with GENECLAN, see example 7) then ligated into the XmaI-BamHI cut pNG2 plasmid to give pNG3.

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The pNG4 vector was prepared as follows. The pNG3 vector was further modified such that the SacI restriction enzyme recognition site in the cloned CMV promoter fragment was corrupted by changing the DNA sequence. This was achieved by the use of a two step PCR mutagenesis reaction using the pNG3 vector as a template. The PCR used two complementary oligonucleotide primers (SEQ ID NOS: 38 and 39) to mutate the Sac I recognition sequence and 2 flanking primers (SEQ ID NOS: 40 and 41) for product amplification. Two Primer pairs (SEQ ID NOS: 38 and 41) and (SEQ ID NOS: 39 and 40) were used in a standard PCR reaction (as described in Example 7, section 7.4) to obtain the initial 2 PCR products, which were isolated by electrophoresis on 2% agarose gels.

Equimolar amounts of each product were mixed and reamplified using the flanking primers (SEQ ID NOS: 40 and 41) under the standard PCR reaction conditions to splice together and amplify the final PCR product. This product was subsequently digested with the restriction enzymes NcoI and HindIII and cloned into the appropriately restricted and prepared pNG3 vector such that the mutated (SacI site minus) fragment replaced the original pNG3 NcoI-Hind III (SacI site plus) fragment. This new vector was named pNG4.

A clone of the 806.077 murine light chain in the Bluescript KS+ vector (VK4) was taken and amplified using the oligonucleotide primers 077VK-UP (SEQ ID NO: 12) and 077VK-DOWN (SEQ ID NO: 13). Similarly a 806.077 heavy chain clone (VH14A) was amplified using 077VH-UP (SEQ ID NO: 14) and 077VH-DOWN (SEQ ID NO: 15). The PCR was performed as follows: To 100ng of plasmid DNA was added 5µl dNTPs (2.5mM), 5µl 10x Enzyme buffer (see above), 1µl of 25pM/µl back primer, 1µl of 25pM/µl forward primer, 0.5µl thermostable DNA polymerase and DEPC-treated water to obtain a volume of 50µl. The PCR conditions were set for 15 cycles at 94°C for 90s; 55°C for 60s; 72°C for 120s, ending the last cycle with a further 72°C for 10 min incubation. The products were analysed on a 2% agarose gel. The DNA was purified and the DNA fragment digested with the relevant restriction enzymes in preparation for subsequent vector cloning.

For secretion of antibody light chain, a double stranded DNA cassette which contained both the information for a Kozak recognition sequence and a light chain signal sequence was designed. The cassette consisted of two individual oligonucleotides (SEQ ID NOS: 42 and 43) which were hybridised and subsequently cloned between the HindIII and SacII restriction site of the pNG3 plasmid (which had been appropriately restricted and isolated using standard methodology) to create the vector pNG3-Vkss. The DNA sequence of

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SEQ ID NO: 46, which contains the sequence for the human light chain kappa constant region, was digested with XmaI and XhoI and inserted between the XhoI and XmaI cut pNG-Vkss plasmid to give the vector pNG3-Vkss-HuCh (NCIMB no. 40798). Furthermore, a neomycin resistance gene expression cassette was cloned into pNG3-Vkss-HuCh (from the pSG5 plasmid variant pSG5-Neo vector, supplied from S.Green, Zeneca Pharmaceuticals; alternative sources include vectors such as pMC1neo, Stratagene cat. no. 213201). The neomycin resistance gene expression cassette was cloned as an XbaI fragment and cloned into the XbaI site of the pNG3-Vkss-HuCh and the orientation was checked using restriction enzyme digestion. This gave rise to the plasmid pNG3-Vkss-HuCh-Neo (NCIMB 40799).

10 The light chain gene sequence described above was inserted, in frame, by cloning directly between the SacII and XhoI sites of the pNG3-Vkss HuCh-neo vector. The PCR fragment obtained for the light chain gene was digested with SacII and XhoI restriction enzymes and cloned into the similarly restricted expression vector containing the VK signal and HuCh constant region coding sequences. The chimaeric 806.077 light chain sequence created is

15 shown in SEQ ID NOS: 16 and 17.

Similarly, for secretion of antibody heavy chain, a double stranded DNA cassette which contained both the information for a Kozak recognition sequence and a heavy chain signal sequence was designed. The cassette consisted of two individual oligonucleotides (SEQ ID NOS: 44 and 45) which were hybridised and subsequently cloned between cloned

20 between the HindIII and EcoRI restriction site of the pNG4 plasmid (which had been appropriately restricted and isolated using standard methodology) to create the vector pNG4-VHss. Heavy chain gene sequences could thus be inserted, in frame, by cloning directly between the EcoRI and SacI sites of the pNG4-VHss vector. The DNA sequence of SEQ ID NO: 47, which contains the coding sequence for human heavy chain IgG2CH1' constant

25 region (SEQ ID NOS: 22 and 23) was digested with SacI and XmaI and cloned into pNG4-VHss cut with SacI and XmaI to give the vector pNG4-VHss-HuIgG2CH1' (NCIMB no. 40797). The PCR fragment obtained for the heavy chain gene was digested with EcoRI and SacI restriction enzymes and cloned into the similarly restricted expression vector pNG4-VHss-HuIgG2CH1' containing the VH signal and HuIgG2 CH1' constant region coding

30 sequences. The chimaeric 806.077 HuIgG2 Fd chain sequence created is shown in SEQ ID NOS: 18 and 19.

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In some instances it may be preferable to use other classes of chimaeric heavy chain Fd constructs. To this end, variants of the heavy chain vector are made containing HuIgG1CH1' (SEQ ID NOS: 20 and 21) or HuIgG3CH1' (SEQ ID NOS: 24 and 25) which are substituted for the HuIgG2CH1' (SEQ ID NOS: 22 and 23) gene.

5       The sequences shown in SEQ ID NOS: 46 and 47 are prepared by a variety of methods including those described by Edwards (1987) *Am. Biotech. Lab.* 5, 38-44, Jayaraman et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 4084-4088, Foguet and Lubbert (1992) *Biotechniques* 13, 674-675 and Pierce (1994) *Biotechniques* 16, 708. Preferably, the sequences shown in SEQ ID NOS: 46 and 47 are prepared by a PCR method similar to that  
10 described by Jayaraman et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 4084-4088.

Once the individual heavy and light chain sequences were constructed a heavy chain Fd gene expression cassette (including both promoter and gene was excised as a BglII/SalI fragment and cloned between into the BamHI/SalI sites of the light chain vector to produce a co-expression vector construct. This construct was transfected into NS0 myeloma cells  
15 (ECACC No. 85110503) via standard techniques of electroporation and transfectants selected for the property of G418 resistance, a trait which is carried as a selectable marker on the expression plasmid construct.

Alternatively the complete heavy chain Fd and light chain genes may simply be excised from their respective vectors as HindIII/XmaI fragments and subsequently cloned  
20 into other expression vector systems of choice.

### Example 9

#### **Hybridization test of nucleic acid variations of specific nucleic acid sequences**

##### **9.1     Hybridisation Test**

25       A method for detecting variant nucleic acids containing sequences related to specific 806.077 antibody sequences is described. These variant nucleic acids may be present in a variety of forms such as the DNA from bacterial colonies or the DNA/RNA from eukaryotic cells fixed on to a membrane as described above in the screening of a cDNA library or as fragments of purified nucleic acid separated by gel electrophoresis and then transferred to a  
30 suitable membrane as for the techniques of Northern (Maniatis et al, Chapter 7, p39) or Southern (Maniatis, chapter 9, p31) hybridisation.

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## 9.2 Hybridisation probe

Hybridisation probes may be generated from any fragment of DNA or RNA encoding the specific 806.077 antibody nucleic sequence of interest, more specifically from the variable region, particularly the region encoding CDR3 of this region. A synthetic oligonucleotide or its complementary sequence can be used as a specific probe for the CDR3 encoding region.

A hybridisation probe can be generated from a synthetic oligonucleotide by addition of a radioactive 5' phosphate group from [ $\gamma$ - $^{32}$ P]ATP by the action of T4 polynucleotide kinase. 20 pmoles of the oligonucleotide are added to a 20 $\mu$ l reaction containing 100mM Tris, pH7.5, 10mM MgCl<sub>2</sub>, 0.1mM spermidine, 20mM dithiothreitol (DTT), 7.55 $\mu$ M ATP, 55 $\mu$ Ci [ $\gamma$ - $^{32}$ P]ATP and 2.5u T4 polynucleotide kinase (Pharmacia Biotechnology Ltd, Uppsala, Sweden). The reaction is incubated for 30 minutes at 37°C and then for 10 minutes at 70°C prior to use in hybridisation. Methods for the generation of hybridisation probes from oligonucleotides (chapter 11) or from DNA and RNA fragments (chapter 10) are given in Maniatis. A number of proprietary kits are also available for these procedures.

## 9.3 Hybridisation conditions

Filters containing the nucleic acid are pre-hybridised in 100ml of a solution containing 6x SSC, 0.1% SDS and 0.25% dried skimmed milk (Marvel™) at 65°C for a minimum of 1 hour in a suitable enclosed vessel. A proprietary hybridisation apparatus such as model HB-1 (Techne Ltd) provides reproducible conditions for the experiment.

The pre-hybridisation solution is then replaced by 10ml of a probe solution containing 6xSSC, 0.1% SDS, 0.25% dried skimmed milk (e.g. Marvel™) and the oligonucleotide probe generated above. The filters are incubated in this solution for 5 minutes at 65°C before allowing the temperature to fall gradually to below 30°C. The probe solution is then discarded and the filters washed in 100ml 6xSSC, 0.1% SDS at room temperature for 5 minutes. Further washes are then made in fresh batches of the same solution at 30°C and then in 10°C increments up to 60°C for 5 minutes per wash.

After washing, the filters are dried and used to expose an X-ray film such as Hyperfilm™ MP (Amersham International) at -70°C in a light-tight film cassette using a fast tungstate intensifying screen to enhance the photographic image. The film is exposed for a suitable period (normally overnight) before developing to reveal the photographic image of the radio-active areas on the filters. Related nucleic acid sequences are identified by the

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presence of a photographic image compared to totally unrelated sequences which should not produce an image. Generally, related sequences will appear positive at the highest wash temperature (60°C). However, related sequences may only show positive at the lower wash temperatures (50, 40 or 30°C).

- 5        These results will also depend upon the nature of the probe used. Longer nucleic acid fragment probes will need to be hybridised for longer periods at high temperature but may remain bound to related sequences at higher wash temperatures and/or at lower salt concentrations. Shorter, mixed or degenerate oligonucleotide probes may require less stringent washing conditions such as lower temperatures and/or higher Na<sup>+</sup> concentrations. A  
10 discussion of the considerations for hybridisation protocols is provided in Maniatis (chapter 11).

#### Example 10

##### **Pharmaceutical compositions**

- 15        The following illustrates representative pharmaceutical dosage forms containing 806.077 antibody which may be used for therapy in combination with a suitable prodrug.

##### Injectable solution for ADEPT

- 20        A sterile aqueous solution, for injection, containing per ml of solution:

806.077 antibody - CPG2 conjugate	1.0mg
Sodium acetate trihydrate	6.8mg
Sodium chloride	7.2mg
Tween 20	0.05mg

- A typical dose of conjugate for adult humans is 30mg followed 3 days later by three 1g doses of prodrug administered at hourly intervals. Suitable CPG2 conjugates are any one of those conjugates described in Examples 105 and 106. Conjugates with HCPB may replace the  
25 CPG2 conjugate in the table. Suitable HCPB conjugates are any one of those conjugates described in Examples 48-101.

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Injectable solution for Tumour Immunotherapy

A sterile aqueous solution, for injection, containing per ml of solution:

806.077 antibody - B7 conjugate	1.0mg
Sodium acetate trihydrate	6.8mg
Sodium chloride	7.2mg
Tween 20	0.05mg

5 A typical dose of conjugate for adult humans is 30mg. A suitable conjugate is described in Example 104.

Example 11**Construction of Initial 806.077 humanised antibody heavy and light chain variable****10 region genes**

Firstly an overview of the humanisation strategy is set out in the following text. The purpose of antibody humanisation is to combine the binding site of a non-human antibody into the supporting framework of a human antibody while maintaining the characteristic antigen binding affinity and specificity properties of the parent antibody. The feasibility of  
 15 such antibody engineering is a consequence of the close sequence and structural homology of immunoglobulins from different mammalian species.

In its most basic form the approach involves the transfer of the six hypervariable regions or complementarity determining regions (CDRs) from one antibody Fv region to another, as first described in Jones et al Nature (1986) 321 522-525. However, experience has  
 20 shown that in addition to the CDRs it is often necessary that amino acids in the antibody framework also need to be transferred for the process to be successful since such residues sometimes appear to contact and influence the conformation of the CDR loops.

In the case of the 806.077 antibody an "Initial" humanised version of the antibody was made which comprises the six murine CDRs and a number of framework residue  
 25 substitutions. This construct was used as a template from which further variants (Examples 12-47) were made by introducing additional "murine" residue substitutions. The rest of this Example describes the Initial humanised construct in detail.



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The human antibody heavy chain variable region NEWM (Poljak, R.J. et al (1974) PNAS 71 3440-3444) and the light chain kappa variable region REI (Palm, W and Hilschmann, N. Z. (1975) Physiol. Chem. 356 167-191) were chosen to form the acceptor human antibody framework. Numerous examples of successful humanisations using this Fv framework have been described in the literature and the 3 dimensional structure of these two protein domains has been solved. Based on comparison of the murine 806.077 heavy and light chain variable region protein sequences with their closest related Kabat murine subgroup consensus sequences (and the individual sequence members) and the human NEWM and REI protein sequences, individual DNA sequences were designed to encode for the Initial humanised antibody which incorporated the murine CDRs and any additional framework substitutions considered to be of importance.

The murine 806.077 CDR sequences incorporated are described in SEQ ID NOs: 26; 27 and 28 for the light chain variable region and are found at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3) respectively. The CDRs incorporated in the heavy chain variable region are described in SEQ ID NOs: 29, 31 and 32 being at positions 31-35 (CDR1), 50-65 (CDR2), 95-102 (CDR3) respectively (using Kabat nomenclature). In the heavy chain variable region the additional changes V24A; S27F; T28N; F29I; S30K; V71A; A92H; R93V (Kabat nomenclature) were made to the NEWM framework and in the light chain variable region no additional framework changes were made.

Individual synthetic DNA sequences were designed to encode for the initial version of the 806.077 humanised antibody heavy (806.077HuVH1) and light chain (806.077HuVK1) variable regions in which the CDRs and any additional framework residue changes were incorporated. The antibody variable gene sequences shown in SEQ ID NOS: 48 and 53 may be prepared by a variety of methods including those described by Edwards (1987) Am. Biotech. Lab. 5, 38-44, Jayaraman et al. (1991) Proc. Natl. Acad. Sci. USA 88, 4084-4088, Foguet and Lubbert (1992) Biotechniques 13, 674-675 and Pierce (1994) Biotechniques 16, 708. Preferably, the DNA sequences shown in SEQ ID NOS: 48 and 53 are prepared by a PCR method similar to that described by Jayaraman et al. (1991) Proc. Natl. Acad. Sci. USA 88, 4084-4088.

The humanised 806.077 antibody variable light chain gene sequence (SEQ ID NO: 49 and 50) was inserted, in frame, by cloning into the pNG3-Vkss-HuCK-Neo (NCIMB no. 40799) expression vector. To achieve this, the synthetic PCR DNA fragment encoding the

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humanised variable light chain gene (SEQ ID NO: 48) was digested with SacII and XhoI restriction enzymes and cloned into the similarly restricted pNG3-Vkss-HuCK-Neo vector which contained the VK signal sequence and HuCK constant region coding sequences. The DNA and protein sequence of completed humanised 806.077 light chain sequence

5 (806.077HuVK1-HuCK) produced, together with its signal sequence, are shown in SEQ ID NOS: 51 and 52 respectively and the vector named pNG3-Vkss-806.077HuVK1-HuCK-Neo.

Similarly, for the humanised antibody heavy chain, the humanised variable heavy chain gene sequence SEQ ID NO: 54 and 55 was inserted, in frame, by cloning directly into the pNG4-VHss-HuIgG2CH1' (NCIMB no. 40797) expression vector. To achieve this, the  
10 synthetic PCR fragment obtained for the humanised heavy chain gene (SEQ ID NOS: 53) was digested with EcoRI and SacI restriction enzymes and cloned into the similarly restricted pNG4-VHss-HuIgG2CH1' vector which contained the VH signal sequence and HuIgG2 CH1' constant region coding sequences. The DNA and protein sequence of completed humanised 806.077 Fd heavy chain sequence (806.077HuVH1-HuIgG2 Fd) produced,  
15 together with its signal sequence, are shown in SEQ ID NOS: 56 and 57 respectively and the vector named pNG4-VHss-806.077HuVH1-HuIgG2 CH1'.

The Initial humanised antibody construct was produced by constructing a co-expression plasmid containing both 806.077 HuVK1 light chain and 806.077 HuVH1 heavy chain variable region antibody genes. The plasmid pNG3-Vkss-806.077HuVK1-HuCK-Neo  
20 vector, which contains the humanised light chain variable region HuVK1 (SEQ ID NOS: 49 and 50) was digested using the restriction enzymes BamHI and SalI and the vector run on a 1% agarose gel, the vector band was excised and purified. The plasmid pNG4-VHss-806.077HuVH1-HuIgG2 CH1' (which contains the humanised 806.077HuVH1 heavy chain variable region (SEQ ID NOS: 56 and 57)) was digested using the restriction enzymes BglII  
25 and SalI, the reaction run on a 2% agarose and the fragment band excised and purified. The DNA fragment recovered was subsequently ligated into the prepared pNG3-Vkss-806.077HuVK1-HuCK-Neo vector to produce clones of the desired HuVH1/HuVK1 co-expression vector.

These constructs were transfected into NS0 myeloma cells (ECACC No. 85110503)  
30 via standard techniques of electroporation and transfectants selected for the property of G418 antibiotic resistance. The clones obtained were tested for both antibody expression in the anti-human antibody Fd ELISA and CEA binding ELISA assays described below.

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For the CEA ELISA each well of a 96 well immunoplate (NUNC MAXISORB™) was coated with 50ng CEA in 50 mM carbonate/bicarbonate coating buffer pH9.6 (buffer capsules - Sigma C3041) and incubated at 4°C overnight. The plate was washed three times with PBS+0.05% Tween 20 and then blocked 150µl per well of 1% BSA in PBS + 0.05% Tween 20 for 1 hour at room temperature. The plate was washed as previously described, 100µl of test sample added per well and incubated at room temperature for 2 hours. Again the plate was washed three times with PBS+0.05% Tween 20, 100µl per well of a 1/500 dilution of HRPO-labelled goat anti-human kappa antibody (Sigma A 7164) was added, in 1% BSA in PBS-Tween 20 and incubated at room temperature on a rocking platform for at least 1 hour.

10 The plate was washed as before and then once more with PBS. To detect binding add 100µl per well developing solution (one capsule of phosphate-citrate buffer - Sigma P4922 - dissolved in 100 mls H<sub>2</sub>O to which is added one 30 mg tablet *o*-phenylenediamine dihydrochloride - Sigma P8412) and incubated for up to 15 minutes. The reaction was stopped by adding 75µl 2M H<sub>2</sub>SO<sub>4</sub>, and absorbance read at 490nm.

15 In the anti-human antibody Fd ELISA, each well of a 96 well immunoplate was coated with 1.2µg sheep anti-human Fd antibody (Binding Site PC075) in 50 mM carbonate/-bicarbonate coating buffer pH9.6 (buffer capsules - Sigma C3041) and incubated at 4°C overnight. The plate was washed three times with PBS+0.05% Tween 20 and then blocked with 150µl per well of 1% BSA in PBS + 0.05% Tween 20 for 1 hour at room temperature.

20 The plate was washed as previously described, 100µl of test sample added per well and incubated at room temperature for 2 hours. Again the plate was washed three times with PBS+0.05% Tween 20, 100µl per well of HRPO-labelled goat anti-human kappa antibody (Sigma A 7164) was added in 1% BSA in PBS-Tween 20 and incubated at room temperature on a rocking platform for at least 1 hour. Wash plate as before and then once more with PBS.

25 To detect binding, developing solution etc was added as described above for the CEA binding assay.

The clones found to show the best expression and CEA binding levels were selected for further expansion into 24 well plates and re-tested. The best clone according to these assay criteria was selected and expanded such that a one litre production was undertaken, which

30 was seeded using a 1:10 dilution of a confluent grown culture (i.e. 100mls into 900mls of fresh culture medium) and the grown for a further 14 days. The human F(ab')<sub>2</sub> antibody fragment was then purified from the culture supernatant as described in Example 102.

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Examples 12-38**Further combinations of humanised heavy and light chain variable region gene****variants: Construction of 806.077 Humanised heavy and light variable region variants.**

The Initial humanised 806.077 variable region genes were also used for the subsequent

5 construction of further gene constructs which contained additional murine framework residues. Modifications of the gene sequences were achieved (in the majority of cases) by cassette mutagenesis. In this technique part of the original gene was removed via restriction with two appropriate unique enzymes from the complete plasmid vector and then replaced by a double stranded DNA cassette (consisting of two complementary oligonucleotides

10 hybridised together to form a DNA fragment with the appropriate cohesive ends) by direct ligation into the prepared plasmid thus reconstituting the gene but now containing desired DNA changes. Further combinations of mutations within either the heavy or light chain could be also be produced by simple DNA fragment exchanges between the appropriate variants by utilising the available unique restriction enzyme sites.

15 Three further variants of the humanised light chain variable region were produced in addition to the original sequence HuVK1 (SEQ ID NO: 49 and 50) and these were called HuVK2, HuVK3 and HuVK4 respectively. The light chain variable region variant HuVK2 was a modification of the original HuVK1 coding sequence in order to produce the amino acid change M4L (Kabat nomenclature), with the gene (SEQ ID NO: 49) being mutated by cassette

20 mutagenesis. The plasmid pNG3-Vkss-806.077HuVK1-HuCK-Neo (which contains the complete humanised light chain (SEQ ID NOS: 49 and 50) was digested using the restriction enzymes SacII and NheI. The digest was then loaded on a 2 % agarose gel and the excised fragment separated from the remaining vector. The vector DNA was then excised from the gel, recovered and stored at -20°C until required. Two oligonucleotides (containing the

25 desired base changes) were designed and synthesised (SEQ ID NO: 58 and 59). These two oligonucleotides were hybridised by adding 200 pmoles of each oligonucleotide into a total of 30 µl of H<sub>2</sub>O, heating to 95°C and allowing the solution to cool slowly to 30°C. 100pmoles of the annealed DNA product was then ligated directly into the previously prepared vector. This DNA "cassette" exchange produced the desired HuVK2 DNA and protein sequence

30 (SEQ ID NO: 60 and 61) already in place in the expression vector pNG3-Vkss-806.077HuVK2-HuCK-Neo.

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Similarly, HuVK3 with the amino acid changes D1Q; Q3V; M4L (Kabat nomenclature) was constructed using synthetic oligonucleotides (SEQ ID NO: 62 and 63) to produce the desired HuVK3 DNA and protein sequence (SEQ ID NO: 64 and 65) again already in place in the expression vector pNG3-Vkss-806.077HuVK3-HuCK-Neo.

5       The light chain variable region variant HuVK4 was produced by a different technique, as there were not unique restriction enzyme sites available close to the mutation site. HuVK4, with the amino acid change L47W, was produced by a PCR mutagenesis technique. The vector pNG3-806.077HuVK1-HuVK-Neo was used as the template for two PCR reactions (94°C, 90sec; 55°C, 60 sec; 72°C, 120 sec for 15 cycles, all buffers, etc., as previously  
10 described). Reaction A used the synthetic oligonucleotide sequence primers SEQ ID NOS: 66 and 67 and reaction B the synthetic oligonucleotide sequence primers SEQ ID NOS: 68 and 69. The products of these PCR reactions (A and B) were fragments of length 535 base pairs and 205 base pairs respectively. These reaction products were run on a 2 % agarose gel and separated from any background products. Bands of the expected size were excised from  
15 the gel and recovered. Mixtures of varying amounts of the products A and B were made and PCR reactions performed using the synthetic oligonucleotides SEQ ID NOS: 66 and 68. The resulting product (ca.700 base pairs) was digested with the restriction enzymes SacII and XhoI and the cleavage products separated on a 2% agarose gel. The band of the expected 310 base pairs size was excised from the gel and recovered. This fragment was then ligated into  
20 the vector pNG3-806.077HuVK1-HuVK-Neo vector (which had been previously cut with the restriction enzymes SacII/XhoI and subsequently isolated) and thus created the desired HuVK4 DNA and protein sequence (SEQ ID NO: 70 and 71) within the expression vector pNG3-Vkss-806.077HuVK4-HuCK-Neo.

      Six further variants of the humanised heavy chain variable region were produced in  
25 addition to the original HuVH1 sequence (SEQ ID NO: 54 and 55) and these were called HuVH2 to HuVK7 respectively. The heavy chain variable region variant HuVH2 was a modification of the original HuVH1 coding sequence in order to produce the amino acid change G49A (Kabat nomenclature), with the gene (SEQ ID NO: 54) being mutated by cassette mutagenesis. The plasmid pNG4-VHss-806.077HuVH1-HuIgG2 CH1' (which  
30 contains the complete humanised, IgG2 heavy chain Fd (SEQ ID NOS: 56 and 57) was digested using the restriction enzymes StuI and NotI. The digest was then loaded on a 2 % agarose gel and the excised fragment separated from the remaining vector. The vector DNA

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was then excised from the gel, recovered and stored at -20°C until required. Two oligonucleotides were designed, synthesised (SEQ ID NO: 72 and 73), hybridised and the product ligated directly into the previously prepared vector. This DNA "cassette" exchange produced the desired HuVH2 DNA and protein sequence (SEQ ID NO: 74 and 75) already in place in the expression vector pNG4-VHss-806.077HuVH2-HuIgG2 CH1'.

Similarly, HuVH3 with the amino acid changes T73S; F78A (Kabat nomenclature) was constructed using synthetic oligonucleotides (SEQ ID NO: 76 and 77), however, in this case, the vector pNG4-VHss-806.077HuVH1-HuIgG2 CH1' was digested using the restriction enzymes NotI and SacII. The synthetic DNA cassette was ligated directly into the previously prepared vector to produce the desired HuVH3 DNA and protein sequence (SEQ ID NO: 78 and 79) in the expression vector pNG4-VHss-806.077HuVH3-HuIgG2 CH1'.

HuVH4 with the amino acid changes G49A; T73S; and F78A (Kabat nomenclature) combines the HuVH2 (SEQ ID NO: 74 and 75) and HuVH3 (SEQ ID NO: 78 and 79) variants. This was achieved by digesting the pNG4-VHss-806.077HuVH3-HuIgG2 CH1' vector with the enzymes NotI and NheI and isolating the ca. 200 base pairs NotI/NheI restriction fragment after separation on a 2% agarose gel. The fragment was recovered and subsequently ligated into the pNG4-VHss-806.077HuVH2-HuIgG2 CH1' vector (which had been digested with the same Not I and NheI restriction enzymes and the vector fragment purified). The resulting clones contained the desired HuVH4 DNA and protein sequence ((SEQ ID NO: 80 and 81) in the expression vector pNG4-VHss-806.077HuVH4-HuIgG2 CH1'.

HuVH5 with the amino acid changes V67A (Kabat nomenclature) was constructed using synthetic oligonucleotides (SEQ ID NO: 82 and 83). Again, the vector pNG4-VHss-806.077HuVH1-HuIgG2 CH1' was digested using the restriction enzymes NotI and SacII. The synthetic DNA cassette was ligated directly into the previously prepared vector to produce the desired HuVH5 DNA and protein sequence (SEQ ID NO: 84 and 85) in the expression vector pNG4-VHss-806.077HuVH5-HuIgG2 CH1'.

HuVH6 with the amino acid changes V67A; T73S and F78A (Kabat nomenclature) was constructed using synthetic oligonucleotides (SEQ ID NO: 86 and 87) and for this mutant the vector pNG4-VHss-806.077HuVH1-HuIgG2 CH1' was digested using the restriction enzymes NotI and SacII. The synthetic DNA cassette was ligated directly into the

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previously prepared vector to produce the desired HuVH6 DNA and protein sequence (SEQ ID NO: 88 and 89) in the expression vector pNG4-VHss-806.077HuVH6-HuIgG2 CH1'.

HuVH7 with the amino acid changes G49A; V69A; T73S; and F78A (Kabat nomenclature) combines the HuVH2 (SEQ ID NO: 74 and 75) and HuVH6 (SEQ ID NO: 88 and 89) variants. This was achieved by digesting the pNG4-VHss-806.077HuVH6-HuIgG2 CH1' vector with the enzymes NotI and NheI and isolating the ca. 200 base pairs NotI/NheI restriction fragment after separation on a 2% agarose gel. The fragment was recovered and ligated into the pNG4-VHss-806.077HuVH2-HuIgG2 CH1' vector (which had been digested with the same Not I and NheI restriction enzymes and the vector fragment purified). The 10 resulting clones contained the desired HuVH7 DNA and protein sequence (SEQ ID NO: 90 and 91) in the expression vector pNG4-VHss-806.077HuVH7-HuIgG2 CH1'.

Combinations of such humanised heavy and light chain variable gene variants were made by excising the heavy chain Fd gene variant expression cassette (including both promoter and gene excised as a BglII/SalI fragment) and cloning this fragment into the 15 BamHI/SalI sites of the light chain variant vector to produce a co-expression vector construct. A listing of the possible combinations of variants based on the humanised heavy and light chain variants previously described is shown in the table below.

**Table- Combinations of humanised heavy and light chain variable region variants.**

Example No.	Heavy chain variable region	SEQ ID NOS:	Light chain variable region	SEQ ID NOS:	Co-expression Plasmid Vector
11	HuVH1	54 and 55	HuVK1	49 and 50	pNG 806HuVH1/HuVK1/HuIgG2
12	HuVH1	54 and 55	HuVK2	60 and 61	pNG 806HuVH1/HuVK2/HuIgG2
13	HuVH1	54 and 55	HuVK3	64 and 65	pNG 806HuVH1/HuVK3/HuIgG2
14	HuVH1	54 and 55	HuVK4	70 and 71	pNG 806HuVH1/HuVK4/HuIgG2
15	HuVH2	74 and 75	HuVK1	49 and 50	pNG 806HuVH2/HuVK1/HuIgG2
16	HuVH2	74 and 75	HuVK2	60 and 61	pNG 806HuVH2/HuVK2/HuIgG2
17	HuVH2	74 and 75	HuVK3	64 and 65	pNG 806HuVH2/HuVK3/HuIgG2
18	HuVH2	74 and 75	HuVK4	70 and 71	pNG 806HuVH2/HuVK4/HuIgG2
19	HuVH3	78 and 79	HuVK1	49 and 50	pNG 806HuVH3/HuVK1/HuIgG2
20	HuVH3	78 and 79	HuVK2	60 and 61	pNG 806HuVH3/HuVK2/HuIgG2
21	HuVH3	78 and 79	HuVK3	64 and 65	pNG 806HuVH3/HuVK3/HuIgG2

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22	HuVH3	78 and 79	HuVK4	70 and 71	pNG 806HuVH3/HuVK4/HuIgG2
23	HuVH4	80 and 81	HuVK1	49 and 50	pNG 806HuVH4/HuVK1/HuIgG2
24	HuVH4	80 and 81	HuVK2	60 and 61	pNG 806HuVH4/HuVK2/HuIgG2
25	HuVH4	80 and 81	HuVK3	64 and 65	pNG 806HuVH4/HuVK3/HuIgG2
26	HuVH4	80 and 81	HuVK4	70 and 71	pNG 806HuVH4/HuVK4/HuIgG2
27	HuVH5	84 and 85	HuVK1	49 and 50	pNG 806HuVH5/HuVK1/HuIgG2
28	HuVH5	84 and 85	HuVK2	60 and 61	pNG 806HuVH5/HuVK2/HuIgG2
29	HuVH5	84 and 85	HuVK3	64 and 65	pNG 806HuVH5/HuVK3/HuIgG2
30	HuVH5	84 and 85	HuVK4	70 and 71	pNG 806HuVH5/HuVK4/HuIgG2
31	HuVH6	88 and 89	HuVK1	49 and 50	pNG 806HuVH6/HuVK1/HuIgG2
32	HuVH6	88 and 89	HuVK2	60 and 61	pNG 806HuVH6/HuVK2/HuIgG2
33	HuVH6	88 and 89	HuVK3	64 and 65	pNG 806HuVH6/HuVK3/HuIgG2
34	HuVH6	88 and 89	HuVK4	70 and 71	pNG 806HuVH6/HuVK4/HuIgG2
35	HuVH7	90 and 91	HuVK1	49 and 50	pNG 806HuVH7/HuVK1/HuIgG2
36	HuVH7	90 and 91	HuVK2	60 and 61	pNG 806HuVH7/HuVK2/HuIgG2
37	HuVH7	90 and 91	HuVK3	64 and 65	pNG 806HuVH7/HuVK3/HuIgG2
38	HuVH7	90 and 91	HuVK4	70 and 71	pNG 806HuVH7/HuVK4/HuIgG2

Analogously with Example 11, Example 14 was produced by constructing a co-expression plasmid containing both the 806.077 HuVK4 light chain and the 806.077 HuVH1 heavy chain variable region antibody genes. In this case, the plasmid the pNG3-Vkss-  
5 806.077HuVK4-HuCK-Neo vector, which contains the humanised light chain variable region HuVK1 (SEQ ID NOS: 70 and 71) was digested using the restriction enzymes BamHI and SalI and the vector run on an 1% agarose gel and the vector band purified. The plasmid pNG4-VHss-806.077HuVH1-HuIgG2 CH1' (which contains the humanised 806.077 HuVH1 heavy chain variable region (SEQ ID NOS: 56 and 57) was digested using the restriction  
10 enzymes BglII and SalI, the reaction run on an 2% agarose and the fragment band excised and purified. The DNA fragment recovered was ligated into the prepared pNG3-Vkss-806.077HuVK4-HuCK-Neo vector to produce clones of the desired HuVH1/ HuVK4 co-expression vector.

As described in Example 11, these constructs were transfected into NS0 myeloma  
15 cells (ECACC No. 85110503) via standard techniques of electroporation and transfectants selected for the property of G418 resistance. The clones obtained were tested for both



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antibody expression in anti-human antibody Fd ELISA and CEA binding ELISA assays. Clones found to show the best expression and CEA binding levels were selected, expanded and product expressed. Human F(ab')<sub>2</sub> antibody fragment was then purified from the culture supernatant as described in Example 102.

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Example 39-47**Expression of humanised F(ab')<sub>2</sub> fragments with various classes of human heavy chain constant regions**

Other classes of chimaeric heavy chain Fd constructs may be used. Accordingly, 10 additional variants of the heavy chain vectors have been made which contain either HuIgG1CH1' (SEQ ID NOS: 20 and 21) or HuIgG3CH1' (SEQ ID NOS: 24 and 115), the constant regions of which are substituted for the HuIgG2CH1' gene (SEQ ID NOS: 22 and 23). The vectors created were pNG4-VHss-HuIgG1 CH1' and pNG4-VHss-HuIgG3 CH1' respectively. The heavy chain antibody variable region in question can be excised from the 15 appropriate pNG4-VHss-"VH variable region"-HuIgG2 CH1' plasmid by digestion with EcoRI and SacI restriction enzymes and cloned into the similarly restricted pNG4-VHss-HuIgG1CH1' or pNG4-VHss-HuIgG3 CH1' vector and thus produce a completed heavy chain Fd sequence. As described above, once the individual heavy and light chain sequences are constructed, a heavy chain Fd gene expression cassette (including both promoter and gene 20 can be excised by restriction digestion and the fragment cloned between into the appropriate sites of the light chain vector to produce the final co-expression vector. The table below describes Examples 39-47 in which various heavy and light chain variable regions have been combined with a number of different classes of human heavy chain constant regions.

In Example 44, the vector pNG4-VHss-HuIgG3 CH1' was digested with the 25 restriction enzymes EcoRI and SacI restriction enzymes and the vector fragment isolated as previously described. The HuVH1 heavy chain antibody variable region (SEQ ID NOS: 54 and 55) was excised from the pNG4-VHss-806.077HuVH1-HuIgG2 CH1' plasmid by digestion with EcoRI and SacI restriction enzymes and the fragment cloned into the similarly restricted pNG4-VHss-HuIgG3 CH1' vector to produce a completed humanised IgG3 heavy 30 chain Fd sequence (SEQ ID NOS: 94 and 95) in the completed vector pNG4-VHss-806.077HuVH1-HuIgG3 CH1'. The heavy chain Fd gene expression cassette (including both promoter and gene) was excised as a BglII/SalI fragment and cloned into the BamHI/SalI

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sites of the light chain vector pNG3-Vkss-806.077HuVK1-HuCK-Neo (containing the HuVK1-HuCK humanised light chain SEQ ID NOS: 51 and 52) which had been digested using the restriction enzymes BamHI and Sall . run on an 1% agarose, the vector band purified. This produced a co-expression vector (pNG 806HuVH1/HuVK3/HuIgG3) from which the humanised 806.077HuVH1/HuVK1-HuIgG3/Kappa.Fd antibody fragment could be expressed.

**Table**

Example No.	Humanised heavy chain	SEQ ID NOS	Humanised light chain	SEQ ID NOS	Co-expression Plasmid Vector
39	HuVH1-HuIgG1	92 and 93	HuVK1-HuCK	51 and 52	Png 806HuVH1/HuVK1/HuIgG1
40	HuVH1-HuIgG2	56 and 57	HuVK1-HuCK	51 and 52	pNG 806HuVH1/HuVK1/HuIgG2
41	HuVH1-HuIgG3	94 and 95	HuVK1-HuCK	51 and 52	pNG 806HuVH1/HuVK1/HuIgG3
42	HuVH1-HuIgG1	92 and 93	HuVK3-HuCK	96 and 97	pNG 806HuVH1/HuVK3/HuIgG1
43	HuVH1-HuIgG2	56 and 57	HuVK3-HuCK	96 and 97	pNG 806HuVH1/HuVK3/HuIgG2
44	HuVH1-HuIgG3	94 and 95	HuVK3-HuCK	96 and 97	pNG 806HuVH1/HuVK3/HuIgG3
45	HuVH1-HuIgG1	92 and 93	HuVK4-HuCK	98 and 99	pNG 806HuVH1/HuVK4/HuIgG1
46	HuVH1-HuIgG2	56 and 57	HuVK4-HuCK	98 and 99	pNG 806HuVH1/HuVK4/HuIgG2
47	HuVH1-HuIgG3	94 and 95	HuVK4-HuCK	98 and 99	pNG 806HuVH1/HuVK4/HuIgG3

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In Example 47, the vector pNG4-VHss-HuIgG3 CH1' was digested with EcoRI and SacI restriction enzymes and the vector fragment isolated. The HuVH1 heavy chain antibody variable region (SEQ ID NOS: 54 and 55) was excised from the pNG4-VHss-HuVH1-HuIgG2 CH1' plasmid by digestion with EcoRI and SacI restriction enzymes and the

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fragment cloned into the similarly restricted pNG4-VHss-HuIgG3 CH1' vector. This produces a completed humanised IgG3 heavy chain Fd sequence (SEQ ID NOS: 94 and 95) in the completed vector pNG4-VHss-806.077HuVH1-HuIgG3 CH1'. The heavy chain Fd gene expression cassette (including both promoter and gene) was excised as a BglII/Sall 5 fragment and cloned into the BamHI/Sall sites of the light chain vector pNG3-Vkss-806.077HuVK4-HuCK-Neo vector, (containing HuVK4-HuCK humanised light chain SEQ ID NOS: 98 and 99 ) which had been digested using the restriction enzymes BamHI and Sall, run on an 1% agarose, the vector band purified. This produced a co-expression vector construct pNG 806HuVH1/HuVK4/HuIgG3 from which the humanised HuVH1/HuVK1- 10 HuIgG3/Kappa.Fd antibody fragment could be expressed.

The other Examples shown in the table above were all produced in a similar manner to that described in the Examples 44 and 47. However, in the case of the constructs containing human IgG1, the final co-expression vector construction was made by cloning the heavy chain Fd gene expression cassette (including both promoter and gene) excised as a 15 BglII/BamHI fragment (because there is an internal Sall restriction site in the HuIgG1 CH1' constant region gene) and cloned into the BamHI site of the appropriately prepared light chain vector. In this case the orientation of the heavy chain cassette must be checked. This was achieved by restriction digestion (e.g. with the restriction enzyme Hind III) and agarose gel electrophoresis analysis in which the resulting fragment sizes were viewed relative to 20 comparable fragments from a similarly digested HuIgG2 version (Examples 11-38). When the fragmentation patterns matched for both constructs we could be sure that the heavy chain cassette was in the correct orientation.

As previously described in Example 11, these constructs were transfected into NS0 myeloma cells (ECACC No. 85110503) via standard techniques of electroporation and 25 transfectants selected for the property of G418 resistance. The clones obtained were tested for both antibody expression in the anti-human antibody Fd ELISA and CEA binding ELISA assays and the clones found to show the best expression and CEA binding levels were selected, expanded and grown for gene expression. As before, the human F(ab')<sub>2</sub> antibody fragment was then purified from the culture supernatant as described in Example 102.

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Example 48**Preparation of humanised 806.077 F(ab')<sub>2</sub>-[A248S,G251T,D253K]HCPB fusion protein**

This Example describes the preparation of a gene encoding a humanised Fd heavy chain fragment of 806.077 linked to [A248S,G251T,D253K]HCPB and its co-expression with  
 5 a gene encoding a humanised light chain of 806.077 and a gene encoding the pro domain of human carboxypeptidase B to give the F(ab')<sub>2</sub> protein with a molecule of [A248S,G251T,D253K]HCPB at the C-terminus of each of the heavy chain fragments. The constant and hinge regions of the humanised Fd heavy chain fragment are derived from the human IgG3 antibody isotype. The expressed protein is also referred to as antibody-enzyme  
 10 fusion protein.

*(a) Preparation of a gene encoding humanised Fd heavy chain fragment of 806.077 linked to [A248S,G251T,D253K]HCPB and its cloning into pEE6*

A gene encoding humanised 806.077 Fd linked to [A248S,G251T,D253K]HCPB was generated by PCR from pZEN1921 (Reference Example 2). A first PCR was set up with  
 15 template pZEN1921 (2ng) and oligonucleotides SEQ ID NO: 100 and SEQ ID NO: 101 (100pM of each) in buffer (100μl) containing 10mM Tris-HCl (pH8.3), 50mM KCL, 1.5mM MgCl<sub>2</sub>, 0.125mM each of dATP, dCTP, dGTP and dTTP. The reaction was incubated at 94°C for 5 min then thermostable DNA polymerase (2.5u, 0.5 μl) was added and the mixture overlaid with mineral oil (100μl) and the reaction mixture incubated at 94°C for 1 min, 53°C  
 20 for 1 min and 72°C for 2.5 min for 25 cycles, plus 10 min at 72°C. The PCR product of 536 base pairs was isolated by electrophoresis on a 1% agarose (Agarose type I, Sigma A-6013) gel followed by excision of the band from the gel and isolation of the DNA fragment.

A second PCR was set up with template IgG3-pBSIIKS+ (8.7ng, described in Reference Example 4) and oligonucleotides SEQ ID NO: 102 and SEQ ID NO: 103 and the  
 25 954 base pairs fragment isolated as described above. The products from the 2 PCRs were combined (either at 0.2, 1.0 or 5.0 ng/μl) in PCR buffer as described above. The mixture was incubated for at 94°C for 5 min then 10 cycles at 94°C for 1 min and 63°C for 4 min. Oligos SEQ ID NOS: 101 and 102 (100pM of each) in PCR buffer (50μl) were added. After incubation at 94°C for 3 min, the mixture was further incubated at 94°C for 1.5 min, 53°C for  
 30 2 min and 72°C for 2 min for 25 cycles plus 10 min at 72°C. In this process, the G base at position 508 in SEQ ID NO: 115 was changed to an A base.

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The PCR product of 1434 base pairs was isolated by electrophoresis on a 1% agarose gel, purified and digested with NheI (20u) and XbaI (80u) (New England Biolabs Inc.,) in a total volume of 100µl containing 10mM Tris HCl (pH7.9), 50mM NaCl, 10mM MgCl<sub>2</sub>, 1mM DTT and BSA (100µg/ml) for 4h at 37<sup>0</sup>C. The resulting fragment was again isolated by  
5 electrophoresis on a 1% agarose gel and purified. In a similar digestion, vector pNG4-VHss-806.077huVH1-HulgG2CH1' (10µg; Example 11) was cut with NheI and XbaI then calf intestinal alkaline phosphatase (1µl; New England Biolabs, 10u/µl) was added to the digested plasmid to remove 5' phosphate groups and incubation continued at 37<sup>0</sup>C for a further 30 minutes. Phosphatase activity was destroyed by incubation at 70<sup>0</sup>C for 10 minutes. The  
10 NheI-XbaI cut plasmid was purified from an agarose gel. The NheI-XbaI digested PCR product from above (about 500ng) was ligated with the above cut plasmid DNA (about 200ng) in 20µl of a solution containing 50mM Tris-Hcl (pH7.8), 10mM MgCl<sub>2</sub>, 10mM DTT, 1mM ATP, 50 µg/ml BSA and 400u T4 DNA ligase (New England Biolabs, Inc) at 25<sup>0</sup>C for 4h. A 1µl aliquot of the reaction was used to transform 20µl of competent E. coli DH5α cells.  
15 Transformed cells were plated onto L-agar plus 100µg/ml ampicillin. Potential clones containing the gene for humanised 806.077 Fd-[A248S,G251T,D253K]HCPB were identified by PCR. Each clone was subjected to PCR as described above with oligonucleotides SEQ ID NOS: 104 and 105. A sample (10µl) of the PCR reaction was analysed by electrophoresis on a 1% agarose gel. Clones containing the required gene were identified by the presence of a  
20 512 base pairs PCR product. Clones producing the 512 base pairs band were used for DNA minipreps. The DNA samples were checked by digestion with HindIII and XbaI for the presence of 3751 base pairs and 1862 base pairs fragments. Clones containing these fragments on digestion of the DNA with HindIII and XbaI were used for large scale plasmid DNA preparation and the sequence of the insert confirmed by DNA sequence analysis. The  
25 sequence of the expected insert is shown in SEQ ID NO: 112. Of the clones examined above, 2 contained the expected sequence but with a single base mutation. Clone 54 (also designated pMF195) had an T base at position 605 in SEQ ID NO: 112 in place of the A base, whereas clone 68 (also designated pMF198) had a C base at position 1825 instead of the expected T base. The sequence shown in SEQ ID NO: 112 was prepared from pMF195 and and pMF198  
30 by digesting both (10 µg of each) with XmaI (10u) and XbaI (100u) (New England Biolabs) in buffer (100µl) containing 20mM Tris acetate (pH7.9) 50mM potassium acetate, 10mM Mg acetate, 1mM DTT and BSA (100µg/ml). The 215 base pairs fragment from pMF195 and the

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vector fragment from pMF198 (following treatment with alkaline phosphatase) were isolated from a 1% agarose gel and ligated together as described previously. The ligation mix was used to transform competent DH5 $\alpha$  cells. The transformed cells were plated onto L agar plus ampicillin and resulting colonies screened by digestion of the DNA with XmaI and XbaI for the presence of 5400 base pairs and 215 base pairs fragments. Positive clones were used for large scale plasmid DNA preparation and the sequence of the insert confirmed by DNA sequence analysis. The plasmid containing the 806.077 Fd-[A248S,G251T,D253K]HCPB gene from clone number 102 was named pMF213. The HindIII-XbaI fragment from pMF213 was cloned into pEE6 [this is a derivative of pEE6.hCMV - Stephens and Cockett (1989) Nucleic Acids Research 17, 7110 - in which a HindIII site upstream of the hCMV promoter has been converted to a BglII site] in DH5 $\alpha$  (screened by PCR with oligonucleotides SEQ ID NOS: 106 and 107 for a 2228 base pairs insert) to give pMF221.

*(b) Preparation of a co-expression vector for expression of antibody-enzyme fusion protein*

To generate vectors capable of expressing the antibody-enzyme fusion protein in eukaryotic cells, the GS-System<sup>TM</sup> (Celltech Biologics) was used (WO 87/04462, WO 89/01036, WO 86/05807 and WO 89/10404). The procedure requires cloning the humanised antibody light chain gene into the HindIII-XmaI region of vector pEE14. This vector is described by Bebbington in METHODS: A Companion to methods in Enzymology (1991) 2, 136-145. To construct the expression vector, plasmids pEE14 and pNG3-VKss-806.077HuVK4-HuCK-Neo (Example 14) were digested with HindIII and XmaI as described above. The appropriate vector (from pEE14) and insert (732 base pairs from pNG3-VKss-806.077HuVK4-HuCK-Neo) from each digest were isolated from a 1% agarose gel and ligated together and used to transform competent DH5 $\alpha$  cells. The transformed cells were plated onto L agar plus ampicillin (100 $\mu$ g/ml). Colonies were screened by restriction analysis of isolated DNA for the presence of a 732 base pairs fragment on digestion of the DNA with HindIII and XmaI. Clones producing a 732 base pairs restriction fragment were used for large scale plasmid DNA preparation and the sequence of the insert confirmed by DNA sequence analysis. The plasmid containing the humanised light chain sequence of SEQ ID NO: 70 in pEE14 was named pEE14-806.077HuVK4-HuCK.

To make the co-expression vector, pMF221 (10 $\mu$ g) was cut with BglII (20u) and SalI (40U) in buffer (100 $\mu$ l) containing 10mM Tris-HCl (pH 7.9), 150mM NaCl, 10mM

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MgCl<sub>2</sub>, 1mM DTT and BSA (100µg/ml) and the 4560 base pairs fragment isolated by agarose gel electrophoresis and purified. Similarly, pEE14-806.077HuVK4-HuCK was cut with BamHI (40u) and Sall (40u) and the 9.95kb vector fragment isolated and ligated to the BglII-Sall fragment from pMF221 and cloned into DH5α. Colonies were screened by PCR with 2 sets of oligonucleotides (SEQ ID NOS: 104 and 105, and SEQ ID NOS: 108 and 109). Clones giving PCR products of 185 base pairs and 525 base pairs respectively were characterised by DNA sequencing. A clone with the correct sequence was named pMF228 - light chain/Fd-mutant HCPB co-expression vector in DH5α. The humanised Fd-mutant HCPB sequence is shown in SEQ ID NO: 113. Residues 1 to 19 are the signal sequence, residues 20 to 242 are the humanised variable and IgG3 CH1 region, residues 243 to 306 are the IgG3 hinge region and residues 307 to 613 are the mutant HCPB sequence with the changes at residues 248, 251 and 253 from the human HCPB sequence. The changes in the HCPB sequence occur in SEQ ID NO: 113 at positions 554 (Ser), 557 (Thr) and 559 (Lys) respectively.

15 (c) *Preparation of a vector for expression of the pro domain of proHCPB*

A second eukaryotic expression plasmid, pEE12 containing a gene for the prepro sequence, for secretion of the pro domain with an additional C-terminal leucine residue (termed pro-L), of preproHCPB was prepared as described in Reference Example 17 of International Patent Application Number WO 96/20011. Plasmid pMF161 was prepared by PCR from pMF18 as described for the unmodified prepro sequence, but using oligonucleotides SEQ ID NOS: 110 and 111. The 359 base pairs fragment was cloned into pBluescript to give pMF141 and subsequently into pEE12 to give pMF161. The protein sequence of pro-L is shown in SEQ ID NO: 114.

(d) *Expression of antibody-enzyme fusion protein in eukaryotic cells*

25 For expression in eukaryotic cells, vectors containing genes capable of expressing the antibody enzyme-fusion protein (pMF228) and the pro-L sequence (pMF161) were co-transfected into COS-7 cells. COS cells are an African green monkey kidney cell line, CV-1, transformed with an origin-defective SV40 virus and have been widely used for short-term transient expression of a variety of proteins because of their capacity to replicate circular plasmids containing an SV40 origin of replication to very high copy number. There are two widely available COS cell clones, COS-1 and COS-7. The basic

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methodology for transfection of COS cells is described by Bebbington in Methods: A Companion to Methods in Enzymology (1991) 2, p. 141. For expression of HCPB, the plasmid vectors pMF48 and pMF67 (2µg of each) were used to transfect the COS-7 cells ( $2 \times 10^5$ ) in a six-well culture plate in 2ml Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated foetal calf serum (FCS) by a method known as lipofection - cationic lipid-mediated delivery of polynucleotides [Felgner et al. in Methods: A Companion to Methods in Enzymology (1993) 5, 67-75]. The cells were incubated at 37°C in a CO<sub>2</sub> incubator for 20h. The mix of plasmid DNA in serum-free medium (200µl) was mixed gently with LIPOFECTIN™ reagent (12µl) and incubated at ambient temperature for 15min. The cells were washed with serum-free medium (2ml). Serum-free medium (600µl) was added to the DNA/LIPOFECTIN™ and the mix overlaid onto the cells which were incubated at 37°C for 6h in a CO<sub>2</sub> incubator. The DNA containing medium was replaced with normal DMEM containing 10% FCS and the cells incubated as before for 72h. Cell supernatants (diluted 1:10 with 0.025M Tris-HCl pH7.5; 125µl) were analysed for activity against Hipp-Glu (5h assay, in a total volume of 250µl) essentially as described in Example 103. The diluted supernatant resulted in 18.4% hydrolysis of the Hipp-Glu substrate.

Alternatively, the unmodified pro domain (from plasmid pMF67 described in Reference Example 17 of International Patent Application Number WO 96/20011) can be used in place of the pro-L expression plasmid in the above experiment.

Large scale expression of proteins from COS cells is described by Ridder et al. (1995) in GENE 166, 273-276 and by Blasey et al. (1996) in CRYOTECHNOLOGY 18, 183-192.

For stable expression in CHO cells, the procedures described by Bebbington in METHODS: A Companion to Methods in Enzymology (1991) 2, 136-145 using GS selection with 25µM and 50µM MSX are followed. Alternatively, lipofection, essentially as described above for transfection of COS cells may also be used to transfect CHO cells. The cells are transfected with a mixture of plasmids pMF228 and pMF161 or pMF228 and pMF67. Supernatants from surviving colonies are screened by CEA ELISA (described in Example 11) and Western analysis (described below) for the presence of a 170kDa band corresponding to the required antibody enzyme fusion protein. Supernatants, suitably diluted, are also screened for enzyme activity as described in Example 103. Colonies



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expressing the desired antibody enzyme fusion protein are cultured at the required scale (see for Example the publication by M E Reff (1993) in Current Opinion in Biotechnology 4, 573-576 and references cited therein) and fusion protein purified from cell culture supernatant by one or more of the methods described in Example 102.

#### 5 (e) Western analysis

Western blot analysis was performed as described as follows. Aliquots (20 $\mu$ l) of each supernatant sample were mixed with an equal volume of sample buffer (62.5mM Tris, pH6.8, 1% SDS, 10% sucrose and 0.05% bromophenol blue) with and without reductant. The samples were incubated at 65°C for 10 minutes before electrophoresis on a 10 8-18% acrylamide gradient gel (EXCEL™ gel system from Pharmacia Biotechnology Products) in a MULTIPHOR™ II apparatus (LKB Produkter AB) according to the manufacturer's instructions. After electrophoresis, the separated proteins were transferred to a membrane (HYBOND™ C-Super, Amersham International) using a NOVABLOT™ apparatus (LKB Produkter AB) according to protocols provided by the manufacturer.

15 After blotting, the membrane was air dried.

The presence of antibody fragments was detected by the use of an anti-human kappa antibody (Sigma A7164, goat anti-human Kappa light chain peroxidase conjugate) used at 1:2500 dilution. The presence of human antibody fragments was visualised using a chemiluminescence system (ECL™ detection system, Amersham International).

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#### Examples 49-74

##### **Preparation of other humanised 806.077 F(ab')<sub>2</sub>-mutant HCPB fusion proteins**

These Examples describe preparation of genes encoding humanised Fd heavy chain fragments of 806.077 linked to a mutant HCPB (D253K; G251T,D253K; 25 A248S,G251T,D253K) and their co-expression with a gene encoding a humanised light chain of 806.077 and a gene encoding the pro domain of human carboxypeptidase B to give the F(ab')<sub>2</sub> protein with a molecule of mutant HCPB at the C-terminus of each of the heavy chain fragments. The constant and hinge regions of of the humanised Fd heavy chain fragment are derived from the human IgG1 or IgG2 or IgG3 antibody isotype. The expressed proteins are 30 also referred to as antibody-enzyme fusion proteins.

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The procedures described in Example 48 are repeated with the appropriate sequences derived from the table shown below. Oligonucleotides for PCR constructions and clone screening are readily derived from the appropriate sequences.

To change the mutant HCPB sequence, the PCR template, plasmid pZEN1921, in part 5 (a) of Example 48 is replaced with pZEN1860 for [G251T,D253K]HCPB (described in Reference Example 1) or pICI1713 for [D253K]HCPB (described in International Patent Application Number WO 96/20011).

To change the antibody heavy chain constant and hinge region, the PCR template, vector IgG3-pBSIIKS+, in part (a) of Example 48 is replaced with pNG4-VHss-HuIgG1CH1' 10 (described in Examples 39-47) or pNG4-VHss-HuIgG2CH1' (NCIMB No. 40797).

To change the humanised antibody light chain sequence, the vector pEE14-806.077HuVK4-HuCK in part (b) of Example 48 is replaced with pEE14-806.077HuVK1-HuCK or pEE14-806.077HuVK3-HuCK. The vectors pEE14-806.077HuVK1-HuCK and pEE14-806.077HuVK3-HuCK are prepared as described for pEE14-806.077HuVK4-HuCK 15 in part (b) of Example 48 but using the 732 base pairs HindIII-XmaI fragment from pNG-VHss-806.077HuVK1-Neo and pNG-VHss-806.077HuVK3-Neo respectively (described in Examples 12-38) in place of the HindIII-XmaI fragment from pNG-VHss-806.077HuVK4-Neo.

Antibody-enzyme fusion protein variants for each Example are shown in the table 20 below.

Table

Example No.	Humanised Heavy chain	Humanised Light chain	Mutant HCPB Enzyme
49	HuVH1-HuIgG3	HuVK4-HuCK	[D253K]HCPB
50	HuVH1-HuIgG3	HuVK4-HuCK	[G251T,D253K]HCPB
51	HuVH1-HuIgG3	HuVK1-HuCK	[A248S,G251T,D253K]HCPB
52	HuVH1-HuIgG3	HuVK1-HuCK	[D253K]HCPB
53	HuVH1-HuIgG3	HuVK1-HuCK	[G251T,D253K]HCPB
54	HuVH1-HuIgG3	HuVK3-HuCK	[A248S,G251T,D253K]HCPB
55	HuVH1-HuIgG3	HuVK3-HuCK	[D253K]HCPB
56	HuVH1-HuIgG3	HuVK3-HuCK	[G251T,D253K]HCPB
57	HuVH1-HuIgG1	HuVK4-HuCK	[A248S,G251T,D253K]HCPB

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58	HuVH1-HulgG1	HuVK4-HuCK	[D253K]HCPB
59	HuVH1-HulgG1	HuVK4-HuCK	[G251T,D253K]HCPB
60	HuVH1-HulgG1	HuVK1-HuCK	[A248S,G251T,D253K]HCPB
61	HuVH1-HulgG1	HuVK1-HuCK	[D253K]HCPB
62	HuVH1-HulgG1	HuVK1-HuCK	[G251T,D253K]HCPB
63	HuVH1-HulgG1	HuVK3-HuCK	[A248S,G251T,D253K]HCPB
64	HuVH1-HulgG1	HuVK3-HuCK	[D253K]HCPB
65	HuVH1-HulgG1	HuVK3-HuCK	[G251T,D253K]HCPB
66	HuVH1-HulgG2	HuVK4-HuCK	[A248S,G251T,D253K]HCPB
67	HuVH1-HulgG2	HuVK4-HuCK	[D253K]HCPB
68	HuVH1-HulgG2	HuVK4-HuCK	[G251T,D253K]HCPB
69	HuVH1-HulgG2	HuVK1-HuCK	[A248S,G251T,D253K]HCPB
70	HuVH1-HulgG2	HuVK1-HuCK	[D253K]HCPB
71	HuVH1-HulgG2	HuVK1-HuCK	[G251T,D253K]HCPB
72	HuVH1-HulgG2	HuVK3-HuCK	[A248S,G251T,D253K]HCPB
73	HuVH1-HulgG2	HuVK3-HuCK	[D253K]HCPB
74	HuVH1-HulgG2	HuVK3-HuCK	[G251T,D253K]HCPB

**Example 75****Preparation of [A248S,G251T,D253K]HCPB-(humanised 806.077)F(ab')<sub>2</sub> fusion protein**

This Example describes the preparation of a gene encoding pro-

- 5 [A248S,G251T,D253K]HCPB linked to a humanised (version 1 VH with Human IgG3) Fd heavy fragment of antibody 806.077, and its co-expression with a gene encoding a humanised light chain (version 4 VK with CK) of the 806.077 antibody. This gives the F(ab')<sub>2</sub> protein with a molecule of the pro-[A248S,G251T,D253K]HCPB at the N-terminus of each of the heavy chain fragments. The enzyme is activated by the enzymatic removal of the pro domain
- 10 using trypsin.

Standard molecular biology techniques, such as restriction enzyme digestion, ligation, kinase reactions, dephosphorylation, polymerase chain reaction (PCR), bacterial transformations, gel electrophoresis, buffer preparation and DNA generation, purification and isolation, were carried out as described by Maniatis et al., (1989) Molecular Cloning, A

15 Laboratory Manual; Second edition: Cold Spring Harbor Laboratory, Cold Spring Harbor,

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New York, or following the recommended procedures of manufacturers of specific products. In most cases enzymes were purchased from New England BioLabs, but other suppliers, and equivalent procedures may be used. Oligonucleotide sequences were prepared in an Applied Biosystems 380A DNA synthesiser from 5'dimethoxytrityl base-protected nucleoside-2-  
5 cyanoethyl-N,N'-di-isopropyl-phosphoramidites and protected nucleoside linked to controlled-pore glass supports on a 0.2 µmol scale, according to the protocols supplied by Applied Biosystems Inc.

Mutants of HCPB, native HCPB and HCPB fusion proteins were assayed for their ability to convert hippuryl-L-glutamic acid or hippuryl-L-arginine acid to hippuric acid using  
10 an HPLC based assay as described in Example 103 or International Patent Application Number WO 96/20011 Example 20.

Immunoassay techniques were carried out using methods based on those described by Tijssen, (1985) Practice and Theory of Enzyme Immunoassays, Laboratory Techniques in Biochemistry and Molecular Biology Volume 15, Elsevier Science Publishers, Amsterdam, or  
15 following the recommended procedures of manufacturers of specific products.

To generate plasmids capable of expressing the antibody-enzyme fusion protein in eukaryotic cells the GS-System (Celltech Biologics) was used (details in International Patent Application Numbers WO 87/04462, WO 89/01036, WO 86/05807 and WO 89/10404) with the two plasmids pEE6 (a derivative of pEE6.hCMV in which the HindIII restriction site  
20 upstream of the hCMV promoter has been converted to a BglII site {Stephens and Cockett, 1989, Nucleic Acids Research, 17, 7110}) and pEE12 (a derivative of pSV2.GS with a number of restriction sites removed {Bebbington et al, 1992, Bio/Technology, 10, 169}).

*a) Cloning pre-pro-HCPB up to restriction enzyme XmaI cut site (position 1048 in SEQ ID NO: 124)*

25 Double stranded DNA of plasmid pMF18 (as described in International Patent application Number WO 96/20011 Reference Example 19), a construct consisting of pre-pro-HCPB cloned into vector pBluescript II KS+ (Stratagene), was prepared using standard DNA technology (Qiagen plasmid kit or similar), and restriction digested with HindIII and XmaI enzymes, being very careful to ensure complete digestion. Restriction enzyme HindIII cuts  
30 the pMF18 plasmid just prior to the start of the pre-sequence of the HCPB gene. and XmaI cuts at the codon for amino acid 240 (proline) of the mature protein, the HindIII to XmaI

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DNA piece is referred to as the pre-pro-HCPB fragment. DNA of the correct size, containing the pre-pro-HCPB fragment (about 1061 base pairs) was purified.

Double stranded DNA of plasmid vector pUC19 (New England BioLabs) was prepared, restriction digested with HindIII and XmaI, and purified (about 2651 base pairs) in a similar manner to the pre-pro-HCPB fragment. Ligation mixes were prepared to clone the HCPB gene fragment into the pUC19 vector, using a molar ratio of about 1 vector to 2.5 insert, and a final DNA concentration of about 2.5ng/ $\mu$ l, in the presence of T4 DNA ligase, 1mM ATP and enzyme buffer. Following the ligation reaction the DNA mixture was used to transform E.coli strain DH5 $\alpha$ . Cell aliquots were plated on L-agar nutrient media containing 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. A number of colonies were picked and used for mini-preparations of double stranded plasmid DNA. These DNA samples were analysed by restriction enzyme digestion, and a construct of the correct configuration identified. This plasmid containing the pre-pro-HCPB fragment up to the XmaI site in the mature gene is known as pCF003.

15 b) *Cloning [A248S,G251T,D253K]HCPB from position G241 + linker and 5 amino acids of VH*

To separate the HCPB from the Fd sequence a neutral peptide linker consisting of (Glycine-Glycine-Glycine-Serine)<sub>3</sub> was introduced into the sequence during the PCR. In order to generate the fragment of the mutant [A248S,G251T,D253K]HCPB sequence (as documented in Reference Example 2) and add the peptide linker and the first 5 amino acids of the humanised 806.077 VH, a PCR was set up using 100pMols of primers CME 00971 and CME 00972 (SEQ ID NOs: 122 and 123) in the presence of approximately 5ng of pZen1921 DNA, dNTPs to a final concentration of 200 $\mu$ M, Taq polymerase reaction buffer, and 2.5U of Taq polymerase in a final volume of 100 $\mu$ l. The mixture was heated at 94°C for 10 minutes prior to addition to the Taq enzyme, and the PCR incubation was carried out using 30 cycles of 94°C for 1.5 minutes, 55°C for 2 minutes, and 72°C for 2 minutes, followed by a single incubation of 72°C for 10 minutes at the end of the reaction. The PCR product containing the [A248S,G251T,D253K]HCPB fragment (about 298 base pairs) was analysed for DNA of the correct size by agarose gel electrophoresis and found to contain predominantly a band of the correct size. The remainder of the product from the reaction mix was purified and separated from excess reagents using a microconcentrator column (Centricon™ 100, Amicon), followed by DNA isolation by ethanol/sodium acetate precipitation, centrifugation, vacuum drying and

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re-suspension in distilled water. The isolated DNA was restriction digested with enzymes XmaI and EcoRI, and a band of the correct size (about 271 base pairs) purified.

Double stranded DNA of plasmid pCF003 (described above) prepared using standard DNA technology (Qiagen plasmid kits or similar), was restriction digested with XmaI and 5 EcoRI enzymes, and a band of the correct size (about 2696 base pairs) purified.

Ligation mixes were prepared to clone the mutant HCPB gene fragment into the vector, using a molar ratio of about 1 vector to 2.5 insert (1 pCF003 to 2.5 [A248S,G251T,D253K]HCPB fragment PCR product), and a final DNA concentration of about 2.5ng/ $\mu$ l, in the presence of T4 DNA ligase, 1mM ATP and enzyme buffer. Following 10 the ligation reaction the DNA mixture was used to transform E.coli strain DH5 $\alpha$ . Cell aliquots were plated on L-agar nutrient media containing 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. About 200 colonies were picked and plated onto duplicate sterile nitro-cellulose filters (Schleicher and Schull), pre-wet on plates of L-agar nutrient media containing 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and 15 incubated overnight at 37°C. One duplicate plate was stored at 4°C, and acted as a source of live cells for the colonies, the other plate was treated to denature and fix the DNA from the individual colonies to the nitro-cellulose. The nitro-cellulose filter was removed from the agar plate and placed in succession onto filter papers (Whatman) soaked in: 1. 10% SDS for 2 minutes; 2. 0.5M NaOH, 1.5M NaCl for 7 minutes; 3. 0.5M NaOH, 1.5M NaCl for 4 20 minutes; 4. 0.5M NaOH, 1.5M NaCl for 2 minutes; 5. 0.5M Tris pH7.4, 1.5M NaCl for 2 minutes; and 6. 2xSSC (standard saline citrate) for 2 minutes. The filter was then placed on a filter paper (Whatman) soaked in 10xSSC and the denatured DNA was crossed linked to the nitro-cellulose by ultra violet light treatment (Spectrolinker XL-1500 UV crosslinker). The filters were allowed to air dry at room temperature, and were then pre-hybridised at 60°C for 25 one hour in a solution of 6xSSC with gentle agitation (for example using a Techne HB-1D hybridizer). Note pre-hybridisation blocks non-specific DNA binding sites on the filters.

In order to determine which colonies contain DNA inserts of interest the DNA cross-linked to the nitro-cellulose filter was hybridised with a radio-labelled  $^{32}$ P-DNA probe prepared from the [A248S,G251T,D253K]HCPB purified PCR DNA fragment (see above). 30 About 50ng of DNA was labelled with 50 $\mu$ Ci of  $^{32}$ P-dCTP (>3000Ci/mMol) using T7 DNA polymerase in a total volume of 50 $\mu$ l (Pharmacia T7 Quickprime kit), and the reaction allowed to proceed for 15 minutes at 37°C. The labelled probe was heated to 95°C for 2

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minutes, to denature the double stranded DNA, immediately added to 10ml of 6xSSC at 60°C, and this solution was used to replace the pre-hybridisation solution on the filters. Incubation with gentle agitation was continued for about 3 hours at 60°C. After this time the hybridisation solution was drained off, and the filters were washed twice at 60°C in 2xSSC for 5 15 minutes each time. Filters were then gently blotted dry, covered with cling film (Saran™ wrap or similar), and exposed against X-ray film (for example Kodak X-OMAT-ARS™) overnight at room temperature. Following development of the film, colonies containing inserts of interest were identified as those which gave the strongest exposure (darkest spots) on the X-ray film. In this series of experiments about 15% of the colonies gave positive 10 hybridisation. From this 12 colonies were chosen for further screening. These colonies were picked from the duplicate filter, streaked and maintained on L-agar nutrient media containing 100µg/ml ampicillin, and grown in L-broth nutrient media containing 100µg/ml ampicillin.

The selected colonies were used for mini-preparations of double stranded plasmid DNA. These DNA samples were analysed by restriction enzyme digestion, and constructs of 15 the correct configuration identified. In order to ensure that no changes to the DNA sequence had been introduced during the PCR a number of clones with correct restriction mapping were taken for DNA preparation using standard technology (Qiagen plasmid kits or similar), and the inserts sequenced using several separate oligonucleotide primers. A construct of the correct sequence was identified, and this plasmid containing the pre-pro- 20 [A248S,G251T,D253K]HCPB-linker-humanised 806.077 VH gene up to the PstI site (at amino acid 5)(position 1301 in SEQ ID NO: 124) is termed pCF004.

*c. Cloning Humanised 806.077 Fd*

Double stranded DNA of plasmid pNG4-VHss-HuVH1-806.077-IgG3CH1', a construct consisting of the humanised 806.077 version 1 VH with human IgG3 CH1 and 25 hinge region cloned into vector pNG4 (see Example 44), was prepared using standard DNA technology (Qiagen plasmid kit or similar), and restriction digested with PstI and XmaI enzymes. DNA of the correct size, containing the humanised 806.077 Fd fragment (about 854 base pairs) was purified. Double stranded DNA of plasmid vector pUC19 (New England BioLabs) was prepared, restriction digested with PstI and XmaI, and purified (about 2659 30 base pairs) in a similar manner to the humanised 806.077 Fd fragment.

Aliquots of both restricted and purified DNA samples were checked for purity and concentration estimation using agarose gel electrophoresis compared with known standards.

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From these estimates ligation mixes were prepared to clone the humanised 806.077 Fd gene fragment into the pUC19 vector, using a molar ratio of about 1 vector to 2.5 insert, and a final DNA concentration of about 2.5ng/ $\mu$ l, in the presence of T4 DNA ligase, 1mM ATP and enzyme buffer.

- 5        Following the ligation reaction the DNA mixture was used to transform E.coli strain DH5 $\alpha$ . Cell aliquots were plated on L-agar nutrient media containing 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. A number of colonies were picked and used for mini-preparations of double stranded plasmid DNA. These DNA samples were analysed by restriction enzyme digestion, and a construct of the correct configuration
- 10 identified. This plasmid containing the humanised 806.077 Fd fragment from the PstI site to the XmaI site is known as pCF005.

*d) Cloning humanised 806.077 Fd into pre-pro-[A248S,G251T,D253K]HCPB-linker construct*

Double stranded DNA of plasmid pCF005 (as documented above), was prepared using

15 standard DNA technology (Qiagen plasmid kit or similar), and restriction digested with PstI and EcoRI enzymes. DNA of the correct size, containing the humanised 806.077 Fd fragment (about 870 base pairs) was purified. Double stranded DNA of plasmid vector pCF004 (as documented above) was prepared, restriction digested with PstI and EcoRI, and purified (about 3950 base pairs) in a similar manner to the humanised 806.077 Fd fragment.

- 20 Ligation mixes were prepared to clone the humanised 806.077 Fd gene fragment into the pCF004 vector, using a molar ratio of about 1 vector to 2.5 insert, and a final DNA concentration of about 2.5ng/ $\mu$ l, in the presence of T4 DNA ligase, 1mM ATP and enzyme buffer.

Following the ligation reaction, the DNA mixture was used to transform E.coli strain

25 DH5 $\alpha$ . Cell aliquots were plated on L-agar nutrient media containing 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. A number of colonies were picked and used for mini-preparations of double stranded plasmid DNA. These DNA samples were analysed by restriction enzyme digestion, and a construct of the correct configuration identified. This plasmid containing the pre-pro-[A248S,G251T,D253K]HCPB-Linker-

30 Fd(humanised 806.077) in pUC19 is known as pCF006.

*e) Cloning pre-pro-[A248S,G251T,D253K]HCPB-linker-(humanised 806.077)Fd into pEE6 hCMV vector*



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Double stranded DNA of plasmid pCF006 (as documented above), was prepared using standard DNA technology (Qiagen plasmid kit or similar), and restriction digested with HindIII and EcoRI enzymes. DNA of the correct size, containing the fusion protein (about 2185 base pairs) was purified.

5 Double stranded DNA of plasmid vector pEE6 (as documented above) was prepared, restriction digested with HindIII and EcoRI, and purified (about 4775 base pairs) in a similar manner to the fusion protein. Ligation mixes were prepared to clone the humanised 806.077 Fd fusion protein into the pEE6 vector, using a molar ratio of about 1 vector to 2.5 insert, and a final DNA concentration of about 2.5ng/ $\mu$ l, in the presence of T4 DNA ligase, 1mM ATP  
10 and enzyme buffer. Following the ligation reaction the DNA mixture was used to transform E.coli strain DH5 $\alpha$ . Cell aliquots were plated on L-agar nutrient media containing 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. A number of colonies were picked and used for mini-preparations of double stranded plasmid DNA. These DNA samples were analysed by restriction enzyme digestion, and a construct of the correct  
15 configuration identified. This plasmid containing the pre-pro-[A248S,G251T,D253K]HCPB-Linker-Fd(humanised 806.077) in pEE6 is known as pCF007.

*f) Cloning Humanised 806.077 light chain version 4 into pEE12 vector*

Double stranded DNA of plasmid pNG3-VKss-806.077-HuVK4-HuCK-Neo, a construct consisting of the humanised 806.077 version HuVK4 with human CK cloned into  
20 vector pNG3 (see Examples 12-38), was prepared using standard DNA technology (Qiagen plasmid kit or similar), and restriction digested with HindIII and EcoRI enzymes. DNA of the correct size, containing the humanised 806.077 light chain (about 2022 base pairs) was purified. Double stranded DNA of plasmid vector pEE12 was prepared, restriction digested with HindIII and EcoRI, and purified (about 7085 base pairs) in a similar manner to the  
25 humanised 806.077 light chain. Ligation mixes were prepared to clone the humanised 806.077 light chain into the pEE12 vector, using a molar ratio of about 1 vector to 2.5 insert, and a final DNA concentration of about 2.5ng/ $\mu$ l, in the presence of T4 DNA ligase, 1mM ATP and enzyme buffer. Following the ligation reaction the DNA mixture was used to transform E.coli strain DH5 $\alpha$ . Cell aliquots were plated on L-agar nutrient media containing  
30 100 $\mu$ g/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. A number of colonies were picked and used for mini-preparations of double stranded plasmid DNA. These DNA samples were analysed by restriction enzyme digestion, and a construct of

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the correct configuration identified. This plasmid containing the humanised 806.077 light chain version 4 is known as pCF008/4.

g) *Cloning CMVp-pre-pro-[A248S,G251T,D253K]HCPB-linker-(humanised 806.077)Fd into pCF008/4.*

5 Double stranded DNA of plasmid pCF007 (as documented above), was prepared using standard DNA technology (Qiagen plasmid kit or similar), and restriction digested with BglII and Sall enzymes. Restriction enzyme BglII cuts the pCF007 plasmid prior to the start of the CMV MIE leader, promoter and gene for the fusion protein. Restriction enzyme Sall cuts about 520 base pairs after the stop codons of the mature protein. DNA of the correct size,  
10 containing the fusion protein (about 4844 base pairs) was purified. Double stranded DNA of plasmid vector pCF008/4 was prepared, restriction digested with BamHI and Sall, and purified (about 7436 base pairs) in a similar manner to the fusion protein. Ligation mixes were prepared to clone the [A248S,G251T,D253K]HCPB-linker-(humanised 806.077)Fd fusion gene into the pCF008/4 vector, using a molar ratio of about 1 vector to 2.5 insert, and a  
15 final DNA concentration of about 2.5ng/μl, in the presence of T4 DNA ligase, 1mM ATP and enzyme buffer. Following the ligation reaction the DNA mixture was used to transform E.coli strain DH5α. Cell aliquots were plated on L-agar nutrient media containing 100μg/ml ampicillin as selection for plasmid vector, and incubated overnight at 37°C. A number of colonies were picked and used for mini-preparations of double stranded plasmid DNA. These  
20 DNA samples were analysed by restriction enzyme digestion, and a construct of the correct configuration identified. This plasmid containing genes for pro-[A248S,G251T,D253K]HCPB-Linker-F(ab')<sub>2</sub>(humanised 806.077 antibody) in the GS expression vector pEE12 is known as pCF009 and a plasmid map is shown in Figure 2. The DNA and amino acid sequences of the light chain HuVK4 are shown in SEQ ID NOs: 70 and  
25 71. The DNA sequence of the pre-pro-[A248S,G251T,D253K]HCPB-linker-Fd(Humanised 806.077) is shown in SEQ ID NO: 124 and the corresponding amino acid sequence in SEQ ID NO: 125.

h) *Expression of Pro-[mutant]HCPB-linker-F(ab')<sub>2</sub>(humanised 806.077) from mouse myeloma cells.*

30 The following method has been used for myeloma expression of all (D253K and G251T,D253K and A248S,G251T,D253K) mutant pro-HCPB enzyme fusion proteins.

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The preferred mouse myeloma cell line is NS0 (Galfre and Milstein, 1981, Methods in Enzymol., 73, 3-46), and is available from the European Collection of Animal Cell Cultures, PHLS CAMR, Porton Down, Salisbury, Wiltshire, SP4 0JG (ECACC catalogue number 85110503). These cells were grown in Dulbecco's Modified Eagle Medium (DMEM;

5 Gibco/BRL) containing 10% heat inactivated foetal calf serum (FCS).

For expression of pro-[A248S,G251T,D253K]HCPB-linker-F(ab')<sub>2</sub>(humanised 806.077) two plasmids were used, pCF009 (described above) and pRc/RSV (from Invitrogen, Cat. no. V780-20) which contains the neomycin resistance gene for selection of G418 resistant stable cell lines. About 5µg of each plasmid (from 0.5 to 10µg) were used to

10 transfect approximately  $8 \times 10^6$  NS0 cells by the method of lipofection (Felgner et al., in Methods: A Companion to Methods in Enzymology, 1993, 5, 67-75) which involves the cationic lipid mediated delivery of polynucleotides into eukaryotic cells. The cells were harvested by centrifugation, washed with serum free medium (30ml), resuspended in 800µl of medium and kept at 37°C in a tissue culture flask until the DNA was added. Serum-free

15 medium (450µl) was mixed gently with LIPOFECTIN™ reagent (50µl) and incubated at room temperature for 30 to 45 minutes. This mixture was added to 500µl of medium containing the plasmid DNA mixture (in less than 100µl) and left at room temperature for 15 minutes. Serum free medium (600µl) was added to the plasmid DNA-LIPOFECTIN™ mixture, and the complex added to the cells which were incubated for about 5 hours at 37°C in a CO<sub>2</sub>

20 incubator. The DNA containing medium was then replaced with normal DMEM medium (8ml) containing 10% FCS and the cells incubated overnight. The medium was then again replaced with normal DMEM medium (8ml) containing 10% FCS and the cells incubated as previously without selection for 24 hours. At the end of this period the medium was changed to DMEM containing 10% FCS and G418 selection (1.5 mg/ml), and the cells diluted

25 (between 1 in 4 and 1 in 20) (approximately 0.5 to  $1.5 \times 10^6$  cells per plate) in the same medium into micro-titre wells (150µl per well; 2 or more plates per dilution). The micro-titre plates were incubated for at least two weeks at 37°C in a CO<sub>2</sub> incubator and then checked regularly for viable clone formation.

Media from wells containing single viable clones was taken for testing and replaced

30 with fresh media (containing G418). The removed media was tested for antibody binding to CEA in an ELISA (in the same manner as described in International Patent application Number WO 96/20011 Reference Example 5 part 1, except that the secondary antibody

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solution was changed from anti-mouse to anti-human (goat anti-human Kappa light chain peroxidase conjugate, Sigma A7164). Positive samples for the CEA ELISA were also tested for [A248S,G251T,D253K]HCPB enzyme activity (as described above) following activation (removal of the pro domain from the fusion protein) by trypsin (700µg/ml in 50mM Tris-HCl 5 and 150mM NaCl pH 7.6 at 4°C for 1 hour, the reaction being stopped by the addition of a five fold excess of soy bean trypsin inhibitor). A number of clones were identified which produced media that was positive for both 806.077 antibody binding to CEA and [A248S,G251T,D253K]HCPB enzyme activity. These were further tested by non-reducing Western blot analysis (in the same manner as described in International Patent Application 10 Number WO 96/20011 Reference Example 5 part j, except that the antibody solution is changed from anti-mouse to anti-human (goat anti-human Kappa light chain peroxidase conjugate, Sigma A7164) to identify clones which produce predominately F(ab')<sub>2</sub>(806.077) fusion protein. These clones were then expanded, tested for stable generation of the fusion protein over a number of generations, and the highest producers bulked up and stored frozen 15 in liquid nitrogen using standard technology.

Amplification, high-level expression and fermentation of fusion proteins from NS0 myeloma cells was performed in a similar manner to that described by Bebbington et al. (1992) in Bio/Technology 10, 169-175. Fusion protein was purified, and the pro-sequence removed as described in Example 102.

20

#### Examples 76 to 101

#### **Cloning and expression of other variants of pro-HCPB-Linker-(humanised 806.077)Fd + (humanised 806.077) light chain**

The method for the generation of fusion proteins with other mutants of HPCB was 25 similar to that detailed in Example 75 (above), with the exception that in part b. of Example 75 there was a substitution of [D253K]HCPB or [G251T,D253K]HCPB for [A248S,G251T,D253K]HCPB and the plasmid DNA used in the PCR reaction was pICI1713 (as described in International Patent application Number WO 96/20011, Example 15) or pZEN1860 (Reference Example 1) respectively. After cloning, identification, and sequence 30 confirmation the resulting plasmid containing pre-pro-[D253K]HCPB-linker or pre-pro-[G251T,D253K]HCPB-linker and humanised 806.077 VH gene up to the PstI site (at

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amino acid 5) in the pUC19 vector back ground was used in place of pCF004 in the subsequent cloning reactions.

The method for generation of fusion proteins with other CH1 domains was similar to that detailed in Example 75 (above), with the exception that in part c. of Example 75 there was a substitution of plasmids containing either humanised 806.077 VH version 1 with human IgG1 or IgG2 CH1 and hinge regions in place of 806.077-HuVH1-IgG3CH1' (SEQ ID NOs: 92 and 56 respectively). After cloning, identification, and sequence confirmation the resulting plasmid containing the IgG1 or IgG2 sequence was used in place of pCF005 in the subsequent cloning reactions.

10 The method for generation of fusion proteins with other variants of the humanised 806.077 light chain was similar to that detailed in Example 75 (above), with the exception that in part f. of Example 75 there was a substitution of plasmids containing either humanised 806.077 Lc version 1 or version 3 in place of 806.077-HuVK4-HuCK (SEQ ID NOs: 51 and 96 respectively). After cloning, identification, and sequence confirmation the resulting  
15 plasmid containing the alternative light chain sequence was used in place of pCF008/4 in the subsequent cloning reactions. The fusion protein variants for each Example (76 to 101) are shown in the following table.

Table

Example No.	Humanised Heavy chain	Humanised Light chain	Mutant HCPB Enzyme
76	HuVH1-HuIgG3	HuVK4-HuCK	[D253K]HCPB
77	HuVH1-HuIgG3	HuVK4-HuCK	[G251T,D253K]HCPB
78	HuVH1-HuIgG3	HuVK1-HuCK	[A248S,G251T,D253K]HCPB
79	HuVH1-HuIgG3	HuVK1-HuCK	[D253K]HCPB
80	HuVH1-HuIgG3	HuVK1-HuCK	[G251T,D253K]HCPB
81	HuVH1-HuIgG3	HuVK3-HuCK	[A248S,G251T,D253K]HCPB
82	HuVH1-HuIgG3	HuVK3-HuCK	[D253K]HCPB
83	HuVH1-HuIgG3	HuVK3-HuCK	[G251T,D253K]HCPB
84	HuVH1-HuIgG1	HuVK4-HuCK	[A248S,G251T,D253K]HCPB
85	HuVH1-HuIgG1	HuVK4-HuCK	[D253K]HCPB
86	HuVH1-HuIgG1	HuVK4-HuCK	[G251T,D253K]HCPB
87	HuVH1-HuIgG1	HuVK1-HuCK	[A248S,G251T,D253K]HCPB
88	HuVH1-HuIgG1	HuVK1-HuCK	[D253K]HCPB

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89	HuVH1-HuIgG1	HuVK1-HuCK	[G251T,D253K]HCPB
90	HuVH1-HuIgG1	HuVK3-HuCK	[A248S,G251T,D253K]HCPB
91	HuVH1-HuIgG1	HuVK3-HuCK	[D253K]HCPB
92	HuVH1-HuIgG1	HuVK3-HuCK	[G251T,D253K]HCPB
93	HuVH1-HuIgG2	HuVK4-HuCK	[A248S,G251T,D253K]HCPB
94	HuVH1-HuIgG2	HuVK4-HuCK	[D253K]HCPB
95	HuVH1-HuIgG2	HuVK4-HuCK	[G251T,D253K]HCPB
96	HuVH1-HuIgG2	HuVK1-HuCK	[A248S,G251T,D253K]HCPB
97	HuVH1-HuIgG2	HuVK1-HuCK	[D253K]HCPB
98	HuVH1-HuIgG2	HuVK1-HuCK	[G251T,D253K]HCPB
99	HuVH1-HuIgG2	HuVK3-HuCK	[A248S,G251T,D253K]HCPB
100	HuVH1-HuIgG2	HuVK3-HuCK	[D253K]HCPB
101	HuVH1-HuIgG2	HuVK3-HuCK	[G251T,D253K]HCPB

**Example 102****Purification of proteins containing 806.077 antibody sequences**

5 Purification or enrichment of recombinant  $F(ab')_2$  or antibody-enzyme fusion proteins may be achieved from myeloma cell, CHO cell or COS cell supernatants by several methods, used either singly or together. Purification of murine 806.077  $F(ab')_2$ , chimeric 806.077  $F(ab')_2$  constructs and fully humanised 806.077  $F(ab')_2$  constructs, and antibody-enzyme fusion protein constructs incorporating these  $F(ab')_2$  constructs were achieved by one or more  
10 of several different methods, affinity chromatography or anion exchange chromatography, or protein A / protein G chromatography. These techniques can also be applied to purification of 806.077 antibody - B7 fusions (see Example 104).

**a) Antigen Affinity Chromatography**

Carcinoembryonic antigen (CEA), to which the parent murine 806.077 antibody was  
15 raised, was immobilised on a column (using Pharmacia products). In brief, immobilisation was via a stable ester bond to Sepharose™ High Performance medium, NHS-activated prepacked in columns (HiTrap™); coupling of the CEA to the activated matrix was performed following the standard instructions provided with the product.

**Preparation of a 1ml affinity column.**

20 CEA stock solution (8mg/ml) was first diluted with coupling buffer (0.2M sodium hydrogen carbonate, 0.5M sodium chloride; pH8.3) to a final concentration of 0.5 mg/ml. A

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new column was washed with 6ml of ice-cold 1mM HCl at a flow rate not exceeding 1ml/min. Immediately after, the CEA ligand (1ml at 0.5mg/ml) was injected onto the column. The column was sealed at both ends and left to stand for 30 minutes at room temperature. Excess active groups that had not coupled to the ligand were deactivated and any non-specifically bound ligand was washed out of the column by three rounds of alternating high and low pH washes. The buffers used were 0.5M ethanolamine, 0.5M sodium chloride (pH8.3) and 0.1M sodium acetate, 0.5M sodium chloride (pH 4.0). In each round of washes 6ml of each buffer was washed over the column matrix. Finally, the column was washed into storage buffer (0.05M Na<sub>2</sub>HPO<sub>4</sub>, 0.1% NaN<sub>3</sub>, pH7.0).

10

#### Purification procedure

The cell culture supernatant containing the desired F(ab')<sub>2</sub> or fusion construct e.g. chimeric 806.077 F(ab')<sub>2</sub>, humanised 806.077 F(ab')<sub>2</sub>, or antibody-enzyme fusion protein was diluted 1:1 with phosphate buffered saline (pH 7.2) and passed over the 1ml affinity column at a flow rate of 1ml/min. The column had previously been equilibrated with phosphate buffered saline (pH7.2; 50mM sodium phosphate, 150mM sodium chloride). The column was washed with 10 column volumes of phosphate buffered saline after the cell supernatant had passed over it. Bound F(ab')<sub>2</sub> was eluted with 5 column volumes of 100mM sodium citrate (pH3.0), with 1ml fractions of the eluant being collected. Detection of the eluted F(ab')<sub>2</sub> was achieved by Western blot analysis using a suitable antibody peroxidase conjugate (an anti-human Kappa Light chain -peroxidase conjugate in the case of the fully humanised F(ab')<sub>2</sub>, Sigma A-7164) and developing with hydrogen peroxide and 4-chloro-1-naphthol. Appropriate fractions were pooled and concentrated, using a centrifugal concentrator (Centricon™ 30), where necessary.

#### 25 b) *Anion Exchange Chromatography*

Cell culture supernatant containing the required F(ab')<sub>2</sub> or fusion construct e.g. chimeric 806.077 F(ab')<sub>2</sub>, humanised 806.077 F(ab')<sub>2</sub>, or antibody-enzyme fusion protein was diafiltered into 50 mM Tris (using a stirred cell with a 10,000 molecular weight cut-off membrane) until the ionic strength of the solution was equivalent to the column equilibration buffer. The 40ml aliquot of the diafiltered supernatant was loaded on to a suitable column (Pharmacia Mono Q™ 10/10 HR) at 2ml/min. The column was previously equilibrated with 50mM Tris (pH8.0). Once the supernatant had passed over the column, the column was

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washed back to baseline with the equilibration buffer. Bound material on the column was then eluted with a 0-50% buffer B (50mM Tris, 1M sodium chloride pH8.0 ) over 15 column volumes. Elution fractions were collected (4ml per fraction ) and those containing the  $F(ab')_2$  were identified by Western blot analysis using a suitable antibody peroxidase conjugate (an

5 anti-human Kappa Light chain -peroxidase conjugate in the case of the fully humanised  $F(ab')_2$  , Sigma A-7164) and developing with hydrogen peroxide and 4-chloro-1-naphthol. Appropriate fractions were pooled and concentrated using a centrifugal concentrator (Centricon™ 30), where necessary.

c) *Protein A and Protein G Purification*

10 The cell culture supernatant containing the desired  $F(ab')_2$  or fusion construct (e.g. 806.077  $F(ab')_2$ , chimeric 806.077 IgG<sub>1</sub> or IgG<sub>2</sub> or IgG<sub>3</sub>; pro-HCPB-linker-806.077  $F(ab')_2$  ,806.077  $F(ab')_2$ -HCPB) was diluted 1:1 with phosphate buffered saline before being loaded on to a column previously equilibrated in phosphate buffered saline (pH7.2). The column was washed with phosphate buffered saline, back to baseline, before the bound  $F(ab')_2$  or

15 fusion protein was eluted with 100mM sodium citrate (pH 3.0) in the case of the  $F(ab')_2$  and 50mM glycine, 100mM sodium chloride (pH10.8) in the case of the fusion proteins. Elution fractions were collected and neutralised by the addition of 125µl 2M Tris per 1ml of elution volume. Those fractions containing the  $F(ab')_2$  were pooled and concentrated where necessary using a centrifugal concentrator.

20 d) *Pro-sequence cleavage:*

For fusion proteins containing a covalently linked pro-sequence e.g.(Pro-HCPB-linker-806.077  $F(ab')_2$  ) the pro sequence was cleaved by incubation the fusion with trypsin. This procedure at a milligram (of fusion) scale involved the following. Trypsin was mixed with the fusion protein in a ratio of 1:1000 (trypsin:fusion). The mixture was incubated for 24

25 hours at room temperature (around 22°C), after which the cleavage of the pro sequence was complete. The fusion protein was separated from the pro sequence by recirculating the mixture in one of the generic chromatography purification or enrichment protocols.



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**Example 103****Assay of activity of antibody-enzyme fusion proteins containing mutant human CPB against Hipp-Glu prodrug analogues**

Cell culture supernatants or purified antibody-enzyme fusion proteins containing 5 mutants of human CPB (D253K; G252T,D253K; A248S,G251T,D253K: Examples 48-101) are assayed for their ability to convert hippuryl-L-glutamic acid (Hipp-Glu ; Reference Example 9 in International Patent Application Number WO 96/20011) to hippuric acid using a HPLC based assay.

The reaction mixture (250 µl) contains either 4 µg of purified fusion protein or cell 10 culture supernatant (used either neat or diluted with 0.025M Tris-HCl pH7.5; 125µl) and 0.5 mM Hipp-Glu in 0.025 M Tris-HCL, pH 7.5. Samples are incubated for 5 hr at 37°C. The reactions are terminated by the addition of 250 µl of 30% methanol, 70% phosphate buffer (50mM; pH 6.5), 0.2% trifluoroacetic acid and the amount of hippuric acid generated is quantified by HPLC (using a Hewlett Packard 1090 Series 11 with diode 15 array system).

Samples (50 µl) are injected onto a column (25 cm; HICHROM™ Hi-RPB) and separated using a mobile phase of 15% methanol, 85% phosphate buffer (50mM; pH 6.5) at a flow rate of 1ml/min. The amount of product (hippuric acid) produced is determined from calibration curves generated with known amounts of hippuric acid (Sigma-H6375). 20 Results are expressed as the percentage conversion of substrate into product at 37°C at times ranging from 30min-24h depending on rate of conversion.

For antibody-enzyme fusion proteins with an N-terminal proCPB, the pro domain is first removed by treatment with trypsin (700µg/ml) in 50mM Tris-HCl (pH7.6), 150mM NaCl at 4°C for 1h.

25

**Example 104****Preparation of a human B7.1-humanised 806.077 F(ab')<sub>2</sub> fusion protein (hB7-806)**

As in Reference Example 3, a fusion protein consisting of the signal sequence and extracellular domain of human B7.1 fused directly to the 5' coding region of the humanised 30 806.077 antibody Fd chain is constructed using PCR techniques. A HindIII-NheI fragment is created containing the natural signal sequence and extracellular domain of human B7.1 fused to the V<sub>H</sub> region of a humanised 806.077 antibody heavy chain. This is cloned into a suitable

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vector, for example pNG4-V<sub>H</sub>ss-HuIgG2CH1' or pNG4-V<sub>H</sub>ss-HuIgG3CH1' (see Examples 39-47) (replacing bases 1-423 in Seq.ID NO: 18), to create a human B7.1-humanised 806.077 Fd fusion gene. Co-expression of this fusion with a humanised 806.077 L chain (a suitable vector containing the VK4 version of humanised 806.077 light chain is pCF008/4; see Example 75) is then achieved after construction of a co-expression vector using expression systems such as those described herein. Such a vector is used to transfect NS0 myeloma cells and colonies selected on the presence of CEA binding activity in the culture supernatant. Other humanised sequences are described in Examples 39-47.

The hB7-806 fusion protein is expressed from a suitable cell line and purified using protein-A column as described in Reference Example 3 or one of the methods described in Example 102. It should be noted that purification methods other than protein-A columns are preferred for humanised 806.077 antibody fragments and fusion proteins thereof. The fusion protein can be tested for both antigen and receptor binding properties and T-cell co-stimulatory activity when bound to LS174T cells using assays set out in Reference Example 3.

#### Example 105

##### **Preparation of chimeric and humanised 806.077 F(ab')<sub>2</sub>-CPG2 conjugates**

The procedure described in Example 5 was repeated with the murine F(ab')<sub>2</sub> protein replaced by one of the chimeric versions described in Example 8 or one of the humanised versions described in Examples 39-47.

#### Example 106

##### **Preparation of humanised 806.077 Fab-CPG2 enzyme fusion protein.**

Humanised 806.077 antibody and bacterial CPG2 enzyme fusion protein constructs are constructed using PCR methodology similar to that described for the construction of HuVK4 in Examples 12-38, in which specifically designed primers are used in a PCR reaction to amplify the antibody and enzyme gene components (such that the resulting DNA products contain overlapping complementary sequence) which are then joined via a further "splicing / joining" PCR reaction to make the complete antibody-enzyme fusion gene. The fusion protein is created by joining the 3' end of Fd humanised 806.077 antibody heavy chain gene to the 5' end of the CPG2 structural coding gene to create a Fab-CPG2 fusion protein coding gene. In

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such a construct, the humanised 806.077 antibody heavy chain gene component may be terminated after residue K236 for the HuVH1-HuIgG1 Fd heavy chain (SEQ ID NO: 93), after residue Val 237 for the HuVH1-HuIgG2 Fd heavy chain (SEQ ID NO: 57) or after residue Val 237 heavy chain in the HuVH1-HuIgG3 Fd heavy chain (SEQ ID NO: 95) (thus, 5 in each case, excluding any sequence pertaining to the hinge region) and may be joined to the first CPG2 residue positioned C-terminal to the signal sequence cleavage site (Minton et al (1984) Gene 31, 31-38). However, in order to obtain optimal antibody binding and enzymatic properties, it is also envisaged that it may be desirable to incorporate additional residues at the junction between the two constituent components.

10 The fusion gene is then cloned into a suitable vector, for example pNG4-VHss-HuIgG2CH1' (NCIMB no. 40797), after the appropriate restriction enzyme digestion. isolation of the vector and fusion gene DNA fragment have been made thus replacing the original antibody gene with that of the fusion protein. Co-expression of the fusion with a humanised 806.077 light chain is then achieved after construction of a co-expression vector in a manner 15 analogous to that described in Example 11. The co-expression vector is used to transfect NSO myeloma cells and colonies selected on the presence of CEA and Fd binding activity in the culture supernatant as previously described. The fusion protein can be purified using a Protein-A column and shown to have both antigen and enzymatic properties using standard test methodology.

20

#### Example 107

#### **Further combination of humanised heavy and light chain variable regions based on light chain sequence VK4**

The procedures described in Examples 12-38 are repeated with the humanised light 25 chain variable sequence of VK4 (SEQ ID NO: 71) replaced by the modified sequence in which the tyrosine residue (Tyr) at position 35 of SEQ ID NO: 71 is replaced by a phenylalanine residue (Phe).

30

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Example 108**Further combination of humanised heavy and light chain variable regions based on light chain sequence VK4**

The procedures described in Examples 12-38 are repeated with the humanised light  
5 chain variable sequence of VK4 (SEQ ID NO: 71) replaced by the modified sequence in  
which the phenylalanine residue (Phe at position 72 of SEQ ID NO: 71 is replaced by a  
leucine residue (Leu).

Example 109**10 Further combination of humanised heavy and light chain variable regions based on light chain sequence VK4**

The procedures described in Examples 12-38 are repeated with the humanised light  
chain variable sequence of VK4 (SEQ ID NO: 71) replaced by the modified sequence in  
which the tyrosine residue (Tyr) at position 35 and the phenylalanine residue (Phe) at position  
15 72 of SEQ ID NO: 71 are replaced by a phenylalanine residue (Phe) and a leucine residue  
(Leu) respectively.

Example 110**Combination of humanised heavy chain variable regions and a chimeric light chain  
20 sequence**

The procedures described in Examples 12-38 are repeated with the humanised light  
chain variable sequence of replaced by the chimeric sequence of SEQ ID NO: 17 described in  
Example 8.

**25 Example 111-113****Expression of humanised F(ab')<sub>2</sub> fragments with a modified light chain VK4 variable  
sequence**

The procedures described in Examples 39-47 are repeated with the variable light chain  
sequence described in Example 107 used to make a replacement for the humanised light chain  
30 sequence of SEQ ID NO: 99 in which the tyrosine residue (Tyr) at position 57 of SEQ ID NO:  
99 is replaced by a phenylalanine residue (Phe).

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**Example 111** is the combination of HuVH1-HulG1 and the modified SEQ ID NO: 99 described above.

**Example 112** is the combination of HuVH1-HulG2 and the modified SEQ ID NO: 99 described above.

5       **Example 113** is the combination of HuVH1-HulG3 and the modified SEQ ID NO: 99 described above.

**Example 114-116**

**Expression of humanised F(ab')<sub>2</sub> fragments with a modified light chain VK4 variable**  
10 **sequence**

The procedures described in Examples 39-47 are repeated with the variable light chain sequence described in Example 108 used to make a replacement for the humanised light chain sequence of SEQ ID NO: 99 in which the phenylalanine residue (Phe) at position 94 of SEQ ID NO: 99 is replaced by a leucine residue (Leu).

15       **Example 114** is the combination of HuVH1-HulG1 and the modified SEQ ID NO: 99 described above.

**Example 115** is the combination of HuVH1-HulG2 and the modified SEQ ID NO: 99 described above.

**Example 116** is the combination of HuVH1-HulG3 and the modified SEQ ID NO: 20 99 described above.

**Example 117-119**

**Expression of humanised F(ab')<sub>2</sub> fragments with a modified light chain VK4 variable**  
**sequence**

25       The procedures described in Examples 39-47 are repeated with the variable light chain sequence described in Example 109 used to make a replacement for the humanised light chain sequence of SEQ ID NO: 99 in which the tyrosine residue (Tyr) at position 57 and the phenylalanine residue (Phe) at position 94 of SEQ ID NO: 99 is replaced by a phenylalanine residue (Phe) and leucine residue (Leu) respectively.

30       **Example 117** is the combination of HuVH1-HulG1 and the modified SEQ ID NO: 99 described above.

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**Example 118** is the combination of HuVH1-HuIgG2 and the modified SEQ ID NO: 99 described above.

**Example 119** is the combination of HuVH1-HuIgG3 and the modified SEQ ID NO: 99 described above.

5

**Example 120-122**

**Expression of humanised F(ab')<sub>2</sub> fragments with a chimeric light chain sequence**

The procedures described in Examples 39-47 are repeated with the chimeric light chain sequence described in Example 110 replacing the humanised light chain sequences used 10 in Examples 39-47

**Example 120** is the combination of HuVH1-HuIgG1 and the chimeric light chain sequence described above.

**Example 121** is the combination of HuVH1-HuIgG2 and the chimeric light chain sequence described above.

15 **Example 122** is the combination of HuVH1-HuIgG3 and the chimeric light chain sequence described above.

**Example 123**

**Preparation of humanised fusion protein based on modified light chain VK4 sequence**

20 The procedures described in Example 48 are repeated but with plasmid pEE14-806.077HuVK4-HuCK replaced by a plasmid containing the modified VK4 sequence of Examples 107 and 111 to 113.

**Example 124**

25 **Preparation of humanised fusion protein based on modified light chain VK4 sequence**

The procedures described in Example 48 are repeated but with plasmid pEE14-806.077HuVK4-HuCK replaced by a plasmid containing the modified VK4 sequence of Examples 108 and 114 to 116.

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Example 125**Preparation of humanised fusion protein based on modified light chain VK4 sequence**

The procedures described in Example 48 are repeated but with plasmid pEE14-806.077HuVK4-HuCK replaced by a plasmid containing the modified VK4 sequence of Examples 109 and 117 to 119.

Example 126**Preparation of humanised fusion protein based on a chimeric light chain VK4 sequence**

The procedures described in Example 48 are repeated but with plasmid pEE14-806.077HuVK4-HuCK replaced by a plasmid containing the chimeric light chain sequence of Examples 110 and 120 to 122.

Example 127**Preparation of humanised fusion protein based on modified light chain VK4 sequence**

The procedures described in Example 75 are repeated but with plasmid pCF008/4 replaced by a plasmid containing the modified VK4 sequence of Examples 107 and 111 to 113.

Example 128**Preparation of humanised fusion protein based on modified light chain VK4 sequence**

The procedures described in Example 75 are repeated but with plasmid pCF008/4 replaced by a plasmid containing the modified VK4 sequence of Examples 108 and 114 to 116.

Example 129**Preparation of humanised fusion protein based on modified light chain VK4 sequence**

The procedures described in Example 75 are repeated but with plasmid pCF008/4 replaced by a plasmid containing the modified VK4 sequence of Examples 109 and 117 to 119.

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Example 130**Preparation of humanised fusion protein based on a chimeric light chain VK4 sequence**

The procedures described in Example 75 are repeated but with plasmid pCF008/4 replaced by a plasmid containing the chimeric light chain sequence of Examples 110 and 120 5 to 122.

Reference Example 1**Preparation of gene sequence for [G251T,D253K]HCPB**

The method of cloning [G251T,D253K]HCPB in E.coli was very similar to the 10 method described in International Patent application Number WO 96/20011, Example 15. Again pICI266 was used as the cloning vector, but the starting material for PCR site directed mutagenesis was the [D253K]HCPB gene in plasmid pICI1713 (as described in International Patent Application Number WO 96/20011 Example 15). However, in this case site directed mutagenesis was used during the PCR amplification of the gene to change the codon at amino 15 acid position 251 in the mature gene from Glycine to Threonine (GGC to ACT), the G251T change. Also during the generation of this mutation a number of other mutations were generated at the same (G251) site by using a mixture of oligonucleotides with codon changes at G251. Individual mutant genes were identified following transformation and hybridisation by sequencing across the mutation site, prior to complete gene sequencing. In this example 20 only the oligonucleotide for introducing the G251T mutation will be considered. Two PCR mixtures were prepared, in a manner similar to that described in International Patent application Number WO 96/20011 Example 15. In the first reaction primers were CAN 00402 (SEQ ID NO: 116) and CAN 00734 (SEQ ID NO: 117). In the second reaction primers were CAN 00284 (SEQ ID NO: 118) and CAN 01076 (SEQ ID NO: 119). In both reactions 25 the starting DNA was pICI1713.

Aliquots of the two PCR reactions were analysed for DNA of the correct size (about 750 and 250 base pairs) and estimation of concentration by agarose gel electrophoresis, and found to contain predominantly bands of the correct size. Another PCR was then set up using each of the first two PCR products, with the two end primers {CAN 00402 (SEQ ID NO: 116) 30 and CAN 00284 (SEQ ID NO: 118)}. An aliquot of the PCR product was analysed for DNA of the correct size (about 1000 base pairs) by agarose gel electrophoresis and found to contain predominantly a band of the correct size. The remainder of the product from the reaction mix



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was purified, the isolated DNA restriction digested with enzymes NcoI and EcoRI, and a band of the correct size (about 1000 base pairs) purified in a similar manner to that described in International Patent application Number WO 96/20011 Example 16.

pICI266 double stranded DNA was restriction digested with NcoI and EcoRI enzymes, and DNA of the correct size (about 5600 base pairs) was purified. Aliquots of both restricted and purified vector and insert DNA samples were checked for purity and concentration estimation using agarose gel electrophoresis compared with known standards. From these estimates ligation mixes were prepared to clone the HCPB gene into the pICI266 vector in a similar manner to that described in International Patent application Number WO 96/20011 Example 16.

Following the ligation reaction the DNA mixture was used to transform E.coli strain DH5 $\alpha$ , colonies were picked and tested by hybridisation. A number of the clones were then taken for plasmid DNA preparation, and were sequenced over the region of PCR mutation in order to identify clones with the G251T change in a manner similar to that described in International Patent application Number WO 96/20011 Example 16. From the sequencing results a clone containing a plasmid with the required [G251T:D253K]HCPB gene sequence was selected, and the plasmid called pZEN1860.

#### Reference Example 2

#### **20 Preparation of gene sequence for [A248S,G251T,D253K]HCPB**

The method of cloning [A248S,G251T,D253K]HCPB in E.coli was very similar to the method described in Reference Example 1. The starting material for the PCR site directed mutagenesis was the [G251T,D253K]HCPB gene in plasmid pZEN1860 (described in Reference Example 1) in place of pICI1713. However, in this case site directed mutagenesis was used during the PCR amplification of the gene to change the codon at amino acid position 248 in the mature gene from alanine to serine (GCT to TTC), the A248S change. Two PCR mixtures were prepared, in a manner similar to that described in Reference Examples 1. In the first reaction primers were CAN 00402 (SEQ ID NO: 116) and CAN 00720 (SEQ ID NO: 120). In the second reaction primers were CAN 00284 (SEQ ID NO: 118) and CAN 00726 (SEQ ID NO: 121). In both reactions the starting DNA was pZEN1860.

Methods of PCR, cloning, expression and identification were the same as for Reference Example 1. From the sequencing results a clone containing a plasmid with the

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required [A248S,G251T,D253K]HCPB gene sequence was selected, and the plasmid called pZEN1921.

### Reference Example 3

#### **5 Preparation and characterisation of a human B7.1-murine A5B7 F(ab')<sub>2</sub> fusion protein (AB7)**

Methods for the preparation, purification and characterisation of recombinant murine A5B7 F(ab')<sub>2</sub> antibody have been published (WO 96/20011, Reference Example 5). The cDNA sequence for human B7.1 antigen (also called CD80) has been isolated and described  
10 (Freeman G.J *et al*, Journal of Immunology, 1989, 143, 2714-2722). In this Example "AB7" refers to human B7.1-murine A5B7 F(ab')<sub>2</sub> fusion protein and "A5B7" refers to the anti-CEA antibody termed A5B7.

Using a PCR based strategy we isolated the natural signal sequence and extracellular domain of human B7.1 (encoding amino-acids 1-242) from cDNA prepared from cultured  
15 Raji cells (ATCC No. CCL 86) and fused it directly upstream from the mature 5' coding sequence of the murine A5B7 Fd fragment. This involved isolation of the B7.1 sequence with PCR primers 187/96 and 204/96 (SEQ ID NOS: 126 and 127) and a partial A5B7 Fd sequence with PCR primers 203/96 and 205/96 (SEQ ID NOS: 128 and 129). After purification of the PCR products they were mixed in approximately equimolar amounts and fused by PCR with  
20 primers 187/96 and 205/96. The resulting PCR product was purified, digested with HindIII and BstEII (New England Biolabs (UK) Ltd., Wilbury Way, Hitchin, SG4 OTY) and cloned into the HindIII-BstEII region of pAF1 using standard procedures to create the full length human B7.1-murine A5B7 Fd fusion. This fusion gene (SEQ ID NO: 130 - 131) was cloned as a EcoRI-HindIII fragment into the GS-system<sup>TM</sup> expression vector pEE6 (Celltech  
25 Biologics, Bath Road, Slough, SL1 4EN) according to the protocols described in WO 96/20011, Reference Example 5, to generate vector pAB7.1.

A BglII-SalI fragment containing the B7.1-A5B7 Fd expression cassette was then cloned between the BglII and SalI sites of the vector pAF6 previously described to generate a vector (pAB7.2) capable of co-expressing the fusion protein and the A5B7 L chain. The  
30 vector pAB7.2 was then used to transform NS0 myeloma cells and colonies selected on their ability to grow in the absence of glutamine. Cell lines expressing the fusion protein were identified by determination of CEA binding activity in the culture supernatant using the

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ELISA described. A cell line expressing suitable levels of fusion protein (1D4) was selected for purification and characterisation of the AB7 fusion protein.

*Purification and characterisation of the AB7 fusion protein*

The secreted recombinant B7.1(35-242)-A5B7 F(ab)<sub>2</sub>, AB7, material was purified  
5 from culture supernatant using a Protein-A agarose matrix such as for example Protein-A  
Sephacrose 4 fast flow as manufactured by Pharmacia (Pharmacia Biotech, 23 Grosvenor Rd,  
St Albans, Herts, AL1 3AW). The matrix was washed with 2x 8 matrix volumes of binding  
buffer (3M NaCl, 1.5M Glycine, pH 8.9). The culture supernatant containing AB7 was  
diluted 1:1 with the binding buffer. The washed matrix was added to the diluted culture  
10 supernatant (1ml settled volume of matrix per 40ml of diluted supernatant) and incubated at  
4°C for 2hrs with moderate shaking. The matrix was spun down by centrifugation and  
approx. 75% of the supernatant carefully poured off. The matrix was then resuspended in the  
residual supernatant and the resulting slurry packed into a column. The column was washed  
with 5-6 column volumes of 150mM NaCl, 10mM NaH<sub>2</sub>PO<sub>4</sub>, pH7.4. The buffer was then  
15 changed to 100mM NaCitrate pH2.8 and elution fractions collected. These fractions were  
titrated to approximately pH7.0 by the addition of 2M Tris buffer pH8.5. The elution  
fractions were analysed by non-reducing SDS-PAGE and the peak AB7 fraction(s) retained as  
the product.

*N-Terminal Sequencing*

20 A sample of AB7 was run on reducing SDS-PAGE and blotted onto PVDF  
(polyvinylidene difluoride) membrane (equipment, gels, blotting membrane and methods  
from NOVEX, 4202 Sorrento Valley Blvd, San Diego, CA 92121, USA.). The protein bands  
were stained with Coomassie blue and the band at approximately 70kDa (i.e. B7.1-Fd fusion)  
was N-terminally sequenced (Applied Biosystems, 494 Protein Sequencer (Perkin Elmer, ABI  
25 division, Kelvin close, Birchwood Science Park North, Warrington, WA3 7PB.) The  
sequence obtained matched the expected sequence for mature B7 (ie. after leader sequence  
cleavage from amino-acid 35 in SEQ.ID NO: 131, Val Ile His Val etc.).

*BIAcore Analysis*

AB7 was analysed using BIAcore surface plasmon resonance equipment made by  
30 Biacore (23 Grosvenor Rd, St. Albans, Herts., AL1 3AW, UK.) according to methods for  
BIAcore analysis of the CD80/CTLA-4 interaction taken from Greene JL, Leytze GM,  
Emswiler J, Peach R, Bajorath J, Cosand W, and Linsley PS. (1996) J. Biol. Chem. 271,

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26762-26771. Samples of the purified AB7 product were injected over both a CTLA4-Ig amine coupled surface and a blank (control) amine coupled surface. Binding could clearly be seen to the CTLA4-Ig surface compared to the control surface (see Figure 3). Binding could also be demonstrated between CTLA4-Ig and AB7 when the CTLA4-Ig was injected over an  
5 amine coupled AB7 surface.

Combined with the data from the anti-CEA ELISA these data confirm that the purified AB7 fusion protein has the biological properties of both component parts, namely antigen and receptor binding activities.

*Co-stimulatory activity of the AB7 fusion protein*

10 The ability of the AB7 fusion protein to provide a co-stimulatory signal to T cells when bound to CEA expressing tumour cells was tested using an adaptation of a co-stimulation assay format previously described (Jenkins et al. (1991) J. Immunol. 147:2461). CEA expressing LS174T colo-rectal tumour cells (fixed using 0.5% paraformaldehyde for 5 minutes at room temperature) were incubated with 10µg/ml of the AB7 fusion protein (2  
15 hours rotating at 4°C in RPMI 1640 medium (Gibco, Life Technologies, Paisley, Scotland), containing 0.5% human serum (Sigma AB, Sigma Chemical Co, Dorset, UK.). The cells were washed twice prior to use and binding of the fusion protein confirmed using a fluorescein isothiocyanate (FITC)- conjugated goat-anti-mouse Ig (Becton-Dickinson UK Ltd, Oxford) and flow cytometry (Facsan, Becton Dickinson). To allow the use of unprimed human T  
20 cells in the assay, the T cell receptor (TCR) stimulus was provided by an anti T cell receptor antibody (anti-CD3 antibody, OKT-3 Orthoclinical Diagnostics, Amersham, UK) previously coated onto the wells of a 96 well plate. OKT-3 was immobilised by incubating purified antibody (2µg/ml in bicarbonate coating buffer, pH 9.6 (preformed capsule, Sigma)) overnight at 4° C in 96 well flat bottomed microtitre plates (Costar Corporation, Cambridge, MA, USA),  
25 which were then washed three to four times with PBS. Purified peripheral T cells (from negatively depleted (i.e. pulling out components other than T cells) from donor human blood using magnetic beads (Dynabeads, Dynal A.S, Oslo, Norway) were added to the wells at  $2 \times 10^5$ /well in 50µl of RPMI 1640 medium containing 5% human serum. The fusion protein bound LS174T cells were added to the wells at  $5 \times 10^4$ /well in 50µl of RPMI 1640 medium  
30 plus 5% human serum. Finally the volume in all wells was made up to 200µl using RPMI 1640 medium plus 5% human serum. Cultures were pulsed with 1.25 µCi of [<sup>3</sup>H] thymidine (Amersham International) after 48 hours and harvested 16 hours later with a semi-automated

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cell harvester (TomTec harvester, Wallac UK.). The incorporation of [ $^3$ H] thymidine into DNA was quantitated using liquid scintillation counting (Betaplate Scint and Betaplate counter, Wallac UK.). Data from a typical costimulation assay is displayed in the Table below.

**5 Table: Co-stimulation data**

	$\alpha$ CD3 coated onto wells @ 2 $\mu$ g/ml ( <u>cpm</u> )
T cells alone	3582
T cells + $\alpha$ CD28	28178
T cells + LS174T	12303
T cells + LS174T + $\alpha$ CD28	25759
T cells + LS174T/fusion protein	41755

$\alpha$ CD3 = anti-CD3 antibody;  $\alpha$ CD28 = anti-CD28 antibody

Unprimed T-cells require both T-cell receptor and co-stimulatory signals. In the assay the T-cell receptor signal is provided by  $\alpha$ CD3 antibody. Providing co-stimulation via  $\alpha$ CD28 (Becton-Dickinson used at 0.6 $\mu$ g/ml) stimulates uptake of [ $^3$ H] thymidine over 8 fold compared to  $\alpha$ CD3 alone. The presence of tumour cells has no significant effect on this stimulation. Providing the co-stimulatory signal by AB7 fusion protein bound to tumour cells stimulates uptake of [ $^3$ H] thymidine by more than 3 fold over that given by tumour cells alone and over 11 fold higher than that seen in the absence of co-stimulation. The apparent stimulation provided by tumour cells alone may arise from residual accessory cells in the purified T-cell population. Similar increases in T cell proliferation were consistently observed in wells containing tumour cell bound fusion protein in each of 5 assays carried out compared with wells containing T cells and unbound tumour cells.

#### Reference Example 4

#### **20 Preparation of IgG3-pBSIIKS+**

This example describes the preparation of a vector containing a gene for the human IgG3 heavy chain constant and hinge region.

A gene containing the sequence shown in SEQ ID NO: 115 [this contains a sequence (residues 8 to 508) that is similar to SEQ ID NO: 25, but with residues 312 and 501 of SEQ ID NO: 25 changed to C and G respectively], was prepared by PCR by a method similar to that described by Jayaraman et al. (1991) Proc. Natl. Acad. Sci USA 88, 4084-4088.

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The gene was made in two parts, known as IgG3A and IgG3B. These were cloned separately into the *SacI* and *XmaI* sites of pBluescript KS+ (Stratagene Cloning Systems) to give vectors IgG3A-pBSIIKS+ clone A7 and IgG3B-pBSIIKS+ clone B17 respectively.

IgG3A was made to extend past the *PmaCI* restriction site (CACGTG at positions 334-339 in SEQ ID NO: 115). Similarly, IgG3B was made such that the 5' end of the sequence was upstream of the *PmaCI* restriction site. To obtain the desired IgG3 gene sequence, the intermediate IgG3A and IgG3B vectors were cut with *AflIII* and *PmaCI*. The vector fragment (2823bp) from IgG3A-pBSIIKS+ clone A7, and insert fragment from IgG3B-pBSIIKS+ clone B17 (666bp) were isolated by electrophoresis in a 1% agarose gel and purified. The fragments were ligated and the ligation mix used to transform *E. coli* strain DH5 $\alpha$ . Clones containing the required gene were identified by digestion of isolated DNA with *SacI* and *XmaI* to give a 520bp fragment. The sequence of the insert was confirmed by DNA sequence analysis and clone number F3 was designated IgG3-pBSIIKS+.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

## (i) APPLICANT:

- (A) NAME: ZENECA LIMITED
- (B) STREET: 15 STANHOPE GATE
- (C) CITY: LONDON
- (E) COUNTRY: UNITED KINGDOM
- (F) POSTAL CODE (ZIP): W1Y 6LN
- (G) TELEPHONE: 0171 304 5000
- (H) TELEFAX: 0171 304 5151
- (I) TELEX: 0171 834 2042

10

15

## (ii) TITLE OF INVENTION: PROTEINS

## (iii) NUMBER OF SEQUENCES: 131

20

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

25

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

## (ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGAAGCTTGA AGATGGATAC AGTTGGTGCA GC

32

5 (2) INFORMATION FOR SEQ ID NO: 2:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGAAGCTTAG ACAGATGGGG GTGTCGTTT G

31

25 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 amino acids

(B) TYPE: amino acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Ala  
1 5 10 15  
5  
Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
20 25 30  
Tyr Met  
10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
15 (A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
20 (ii) MOLECULE TYPE: other nucleic acid

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GACATTCAGC TGACCCAGTC TCCA

24

(2) INFORMATION FOR SEQ ID NO: 5:

30

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GACATTGAGC TCACCCAGTC TCCA

24

5 (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

10

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

20 AGGTSMARCT GCAGSAGTCW GG

22

(2) INFORMATION FOR SEQ ID NO: 7:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35

- 93 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: . . . .

ACTAGTGGAA TTCAGTGTGA GGTSCARCTG CAGCARTCWG G

41

5 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 357 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

20 GACATTGAGC TCACCCAGTC TCCAGCAATC ATGTCTGCAT CTCCAGGGGA GAAGGTCACC 60

ATAACCTGCA GTGCCAGCTC AAGTGTAAC TACATGCACT GGTTCAGCA GAAGCCAGGC 120

ACTTCTCCCA AACTCTGGAT TTATAGCACA TCCAACCTGG CTTCTGGAGT CCCTGCTCGC 180

25

TTCAGTGGCA GTGGATCTGG GACCTCTTAC TCTCTCACA TCAGCCGAAT GGAGGCTGAA 240

GATGCTGCCA CTTATTACTG CCAGCAAAGG AGTACTTACC CGCTCACGTT CGGTGCTGGG 300

30 ACCAAGCTGG AGCTGAAACG GGCTGATGCT GCACCAACTG TATCCATCTT CAAGCTT 357

(2) INFORMATION FOR SEQ ID NO: 9:

35

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

15 Asp Ile Glu Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly  
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Tyr Met  
20 25 30

20 His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile Tyr  
35 40 45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser  
50 55 60

25

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu  
65 70 75 80

30 Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Thr Tyr Pro Leu Thr  
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala  
100 105

## 35 (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 base pairs

- 95 -

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: other nucleic acid

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GAGGTGCAGC TGCAGCARTC WGGGGCAGAG CTTGTGAGGT CAGGGGCCTC AGTCAAGTTG 60

TCCTGCACAG CTTCTGGCTT CAACATTAAA GACAACTATA TGCAGTGGGT GAAGCAGAGG 120

15

CCTGAACAGG GCCTGGAGTG GATTGCATGG ATTGATCCTG AGAATGGTGA TACTGAATAT 180

GCCCCGAAGT TCCGGGGCAA GGCCACTTTG ACTGCAGACT CATCCTCCAA CACAGCCTAC 240

20 CTGCACCTCA GCAGCCTGAC ATCTGAGGAC ACTGCCGTCT ATTACTGTCA TGTCTGTATC 300

TATGCTGGTT ATTTGGCTAT GGACTACTGG GGTCAAGGAA CCTCAGTCGC CGTCTCCTCA 360

25 (2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

(B) TYPE: amino acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Ala  
 1                      5                      10                      15  
 5  
 Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
                     20                      25                      30  
 Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile  
 10                      35                      40                      45  
 Ala Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
                     50                      55                      60  
 15  
 Arg Gly Lys Ala Thr Leu Thr Ala Asp Ser Ser Ser Asn Thr Ala Tyr  
 65                      70                      75                      80  
 Leu His Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 20                      85                      90                      95  
 His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln  
                     100                      105                      110  
 25  
 Gly Thr Ser Val Ala Val Ser Ser  
                     115                      120

(2) INFORMATION FOR SEQ ID NO: 12:

30 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 39 base pairs  
     (B) TYPE: nucleic acid  
     (C) STRANDEDNESS: single  
     (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AAGCTTTCCC GCGGGGACAT TGAGCTCACC CAGTCTCCA

39

5 (2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

20 AAGCTTCTCG AGCTTGGTCC CAGCACCGAA

30

(2) INFORMATION FOR SEQ ID NO: 14:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

30 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

AAGCTTGGA TTCAGTGTGA GGTGCAGCTG CAGCAG

36

5 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

20 AAGCTTCGAG CTCACGGCGA CTGAGGTTCC TTG

33

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 705 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid

35



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ATGGATTTC AAGTCAGAT TTTCAGCTTC CTGCTAATCA GTGCTTCAGT CATAATGTCC 60

5 CGCGGGGACA TTGAGCTCAC CCAGTCTCCA GCAATCATGT CTGCATCTCC AGGGGAGAAG 120

GTCACCATAA CCTGCAGTGC CAGCTCAAGT GTAACCTACA TGCACTGGTT CCAGCAGAAG 180

CCAGGCACTT CTCCCAAAC CTGGATTAT AGCACATCCA ACCTGGCTTC TGGAGTCCCT 240

10 GCTCGCTTCA GTGGCAGTGG ATCTGGGACC TCTTACTCTC TCACAATCAG CCGAATGGAG 300

GCTGAAGATG CTGCCACTTA TTACTGCCAG CAAAGGAGTA CTTACCCGCT CACGTTCGGT 360

15 GCTGGGACCA AGCTCGAGAT CAAACGGACT GTGGCTGCAC CATCTGTCTT CATCTTCCCG 420

CCATCTGATG AGCAGTTGAA ATCTGGAAC GCCTCTGTTG TGTGCCTGCT GAATAACTTC 480

TATCCAGAG AGGCCAAAGT ACAGTGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC 540

20 CAGGAGAGTG TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCTG 600

ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT CACCCATCAG 660

25 GGCCTGAGTT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGT 705

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 235 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

	Met	Asp	Phe	Gln	Val	Gln	Ile	Phe	Ser	Phe	Leu	Leu	Ile	Ser	Ala	Ser	
5	1				5					10					15		
	Val	Ile	Met	Ser	Arg	Gly	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro	Ala	Ile	
					20					25					30		
	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Ile	Thr	Cys	Ser	Ala	Ser	
10					35					40					45		
	Ser	Ser	Val	Thr	Tyr	Met	His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Thr	Ser	
					50					55					60		
15	Pro	Lys	Leu	Trp	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	
	65					70					75					80	
	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	
					85					90					95		
20	Ser	Arg	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Arg	
					100					105					110		
	Ser	Thr	Tyr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Ile	Lys	
25					115					120					125		
	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	
					130					135					140		
30	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	
	145					150					155					160	
	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	
					165					170					175		
35	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	
					180					185					190		

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Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu  
195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser  
5 210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
225 230 235

## 10 (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 765 base pairs  
(B) TYPE: nucleic acid  
15 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

25 ATGAAGTTGT GGCTGAACTG GATTTTCCTT GTAACACTTT TAAATGGAAT TCAGTGTGAG 60  
GTGCAGCTGC AGCARTCAGG GGCAGAGCTT GTGAGGTCAG GGCCTCAGT CAAGTTGTCC 120  
TGCACAGCTT CTGGCTTCAA CATTAAAGAC AACTATATGC ACTGGGTGAA GCAGAGGCCT 180  
30 GAACAGGGCC TGGAGTGGAT TGCATGGATT GATCCTGAGA ATGGTGATAC TGAATATGCC 240  
CCGAAGTTCC GGGGCAAGGC CACTTTGACT GCAGACTCAT CCTCCAACAC AGCCTACCTG 300  
35 CACCTCAGCA GCCTGACATC TGAGGACACT GCCGTCTATT ACTGTCATGT CCTGATCTAT 360  
GCTGGTTATT TGGCTATGGA CTACTGGGGT CAAGGAACCT CAGTCGCCGT GAGCTCGGCT 420

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AGCACCAAGG GACCATCGGT CTTCCCCCTG GCCCCCTGCT CCAGGAGCAC CTCCGAGAGC 480

ACAGCCGCCC TGGGCTGCCT GGTCAAGGAC TACTTCCCCG AACCGGTGAC GGTGTCGTGG 540

5 AACTCAGGCG CTCTGACCAG CGGCGTGCAC ACCTTCCCCG CTGTCCTACA GTCCTCAGGA 600

CTCTACTCCC TCAGCAGCGT CGTGACGGTG CCCTCCAGCA ACTTCGGCAC CCAGACCTAC 660

ACCTGCAACG TAGATCACAA GCCCAGCAAC ACCAAGGTGG ACAAGACAGT TGAGCGCAAA 720

10

TGTTGTGTCG AGTGCCCAAC GTGCCCGGCG CCACCTGTGG CCGGC 765

(2) INFORMATION FOR SEQ ID NO: 19:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 255 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Met Lys Leu Trp Leu Asn Trp Ile Phe Leu Val Thr Leu Leu Asn Gly

1 5 10 15

30

Ile Gln Cys Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg

20 25 30

Ser Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile

35

35 40 45

Lys Asp Asn Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu

50 55 60

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	Glu Trp Ile Ala Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala	
	65	70 75 80
5	Pro Lys Phe Arg Gly Lys Ala Thr Leu Thr Ala Asp Ser Ser Ser Asn	
		85 90 95
	Thr Ala Tyr Leu His Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val	
		100 105 110
10	Tyr Tyr Cys His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr	
		115 120 125
	Trp Gly Gln Gly Thr Ser Val Ala Val Ser Ser Ala Ser Thr Lys Gly	
15		130 135 140
	Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser	
	145	150 155 160
20	Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val	
		165 170 175
	Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe	
		180 185 190
25	Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val	
		195 200 205
	Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val	
30		210 215 220
	Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys	
	225	230 235 240
35	Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly	
		245 250 255

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(2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 121 amino acids

5

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

15

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys

1                      5                      10                      15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr

20

20                      25                      30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser

35                      40                      45

25

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser

50                      55                      60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr

65                      70                      75                      80

30

Tyr Ile Cys Asn Val Asn His Asn Pro Ser Asn Thr Lys Val Asp Lys

85                      90                      95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys

35

100                      105                      110

Pro Ala Pro Glu Leu Leu Gly Gly Pro

115                      120

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(2) INFORMATION FOR SEQ ID NO: 21:

5

## (i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 369 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

20

GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCCT CCTCCAAGAG CACCTCTGGG 60  
GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTGCG 120  
25 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA 180  
GGACTCTACT CCCTCAGCAG CGTGGTGACT GTGCCCTCCA GCAGCTTGGG CACCCAGACC 240  
TACATCTGCA ACGTGAATCA CAACCCAGC AACACCAAGG TCGACAAGAA AGTTGAGCCC 300  
30 AAATCTTGTG ACAAGACGCA CACGTGCCCC CCGTGCCCGG CTCCGGAACT GCTGGGTGGC 360  
CCGTAATAG 369

35 (2) INFORMATION FOR SEQ ID NO: 22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 amino acids

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(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg  
1 5 10 15

15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
20 25 30

20

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
50 55 60

25

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr  
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys  
85 90 95

30

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro  
100 105 110

35

Pro Val Ala Gly  
115



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## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 348 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

15

GCTAGCACCA AGGGACCATC GGTCTTCCCC CTGGCCCCCT GCTCCAGGAG CACCTCCGAG 60

AGCACAGCCG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG 120

20 TGGAATCAG GCGCTCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA 180

GGACTCTACT CCTCAGCAG CGTCGTGACG GTGCCCTCCA GCAACTTCGG CACCCAGACC 240

TACACCTGCA ACGTAGATCA CAAGCCCAGC AACACCAAGG TGGACAAGAC AGTTGAGCGC 300

25

AAATGTTGTG TCGAGTGCCC ACCGTGCCCC GCGCCACCTG TGGCCGGC 348

## (2) INFORMATION FOR SEQ ID NO: 24:

30

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 167 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

## (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg  
 1                      5                      10                      15  
 5  
 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
                     20                      25                      30  
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
 10                      35                      40                      45  
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
                     50                      55                      60  
 15  
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr  
 65                      70                      75                      80  
 Tyr Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys  
                     85                      90                      95  
 20  
 Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro  
                     100                      105                      110  
 Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg  
 25                      115                      120                      125  
 Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys  
                     130                      135                      140  
 30  
 Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro  
 145                      150                      155                      160  
 Ala Pro Glu Leu Leu Gly Gly  
                     165  
 35

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- 109 -

- (A) LENGTH: 501 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: other nucleic acid

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
GCTAGCACCA AGGGCCCATC GGTCTTCCCC CTGGCGCCCT GCTCCAGGAG CACCTCTGGG      60
15 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCTG      120
    TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA      180
    GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC      240
20 TACACCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAG AGTGGAGCTG      300
    AAAACCCAC TTGGTGACAC AACTCACACG TGCCCTAGGT GTCCTGAACC TAAATCTTGT      360
    GACACACCTC CCCCGTGCCC ACGGTGCCCA GAGCCCAAAT CTTGCGACAC GCCCCACCG      420
    TGTCCCAGAT GTCCTGAACC AAAGAGCTGT GAACTCCAC CGCCCTGCCC GAGGTGCCCA      480
    GCACCTGAAC TCCTGGGAGG A      501
```

30

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

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(ii) MOLECULE TYPE: peptide

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Ser Ala Ser Ser Ser Val Thr Tyr Met His  
1 5 10

10 (2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

25 Ser Thr Ser Asn Leu Ala Ser  
1 5

(2) INFORMATION FOR SEQ ID NO: 28:

- 30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

5     Gln Gln Arg Ser Thr Tyr Pro Leu Thr  
      1                                5

(2) INFORMATION FOR SEQ ID NO: 29:

10

(i) SEQUENCE CHARACTERISTICS:

15     (A) LENGTH: 5 amino acids  
      (B) TYPE: amino acid  
      (C) STRANDEDNESS: single  
      (D) TOPOLOGY: linear

20     (ii) MOLECULE TYPE: peptide

25     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

      Asp Asn Tyr Met His  
      1                                5

30     (2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

35     (A) LENGTH: 9 amino acids  
      (B) TYPE: amino acid  
      (C) STRANDEDNESS: single  
      (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- 112 -

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Phe Asn Ile Lys Asp Asn Tyr Met His  
1 5

10 (2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

25 Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe Arg  
1 5 10 15

Gly

30

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

35 (B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- 113 -

(ii) MOLECULE TYPE: peptide

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr  
1 5 10

10

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

15

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

25

His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr  
1 5 10

(2) INFORMATION FOR SEQ ID NO: 34:

30

(i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 60 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

TCGAGAGATC TAAGCTTCG CGGGAATTCC TCGAGGAGCT CCCCGGGGGA TCCGTCGACT 60

5

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

20

CTAGAGTCCA CGGATCCCC GGGGAGCTCC TCGAGGAATT CCCGCGGAAG CTTAGATCTC 60

(2) INFORMATION FOR SEQ ID NO: 36:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

AAGCTTCCCG GGTATTAAAG CAGAACTTG

29

5 (2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

20 ACTAGTGGAT CCCAGACATG ATAAGATAC

29

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GGTCTATATA AGCAGAGCTG TCTGGCTAAC TAGAGAACC

39

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(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 39 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GGTTCTCTAG TTAGCCAGAC AGCTCTGCTT ATATAGACC

39

15 (2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

30 GGACTTTCCT ACTTGGCAG

19

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GGCAACTAGA AGGCACAGTC

20

10

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

25

AGCTTGCCGC CACCATGGAT TTTCAAGTGC AGATTTTCAG CTTCTGCTA ATCAGTGCTT

60

CAGTCATAAT GTCCCGC

77

30 (2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid

35

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GGGACATTAT GACTGAAGCA CTGATTAGCA GGAAGCTGAA AATCTGCACT TGAAAATCCA 60

TGGTGGCGGC A 71

10

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 61 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

25

AGCTTGCCGC CACCATGAAG TTGTGGCTGA ACTGGATTTT CCTTGTAACA CTTTAAATG 60

G 61

30 (2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 61 base pairs

(B) TYPE: nucleic acid

35

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

AATTCCATTT AAAAGTGTTA CAAGGAAAAT CCAGTTCAGC CACAAC TTCA TGGTGGCGGC 60

A 61

10

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 357 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

25

AAGCTTCTCG AGATCAAACG GACTGTGGCT GCACCATCTG TCTTCATCTT CCGGCCATCT 60

GATGAGCAGT TGAAATCTGG AACTGCCTCT GTTGTGTGCC TGCTGAATAA CTTCTATCCC 120

30 AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG 180

AGTGTCACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG 240

AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA TCAGGGCCTG 300

35

AGTTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGT T AATAGCCCGG GACTAGT 357

(2) INFORMATION FOR SEQ ID NO: 47:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 381 base pairs

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

15 GGAAGCTTGA GCTCGGCTAG CACCAAGGGA CCATCGGTCT TCCCCCTGGC CCCCTGCTCC 60

AGGAGCACCT CCGAGAGCAC AGCCGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA 120

CCGGTGACGG TGTCGTGGAA CTCAGGCGCT CTGACCAGCG GCGTGACAC CTTCCCGGCT 180

20 GTCCTACAGT CCTCAGGACT CTA TCCCTC AGCAGCGTCG TGACGGTGCC CTCCAGCAAC 240

TTCGGCACCC AGACCTACAC CTGCAACGTA GATCACAAGC CCAGCAACAC CAAGGTGGAC 300

25 AAGACAGTTG AGCGCAAATG TTGTGTCGAG TGCCACCCCT GCCCGGCGCC ACCTGTGGCC 360

GGCTAATAGC CCGGGACTAG T 381

## (2) INFORMATION FOR SEQ ID NO: 48:

30

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

AAGCTTTCCC GCGGCGACAT CCAGATGACC CAGAGCCCAA GCAGCCTGAG CGCTAGCGTG 60  
 5  
 GGTGACAGAG TGACCATCAC GTGTAGTGCC AGCTCAAGTG TAACTTACAT GCACTGGTAC 120  
 CAGCAGAAGC CAGGTAAGGC TCCAAAGCTG CTGATCTACA GCACATCCAA CCTGGCTTCT 180  
 10 GGTGTGCCAA GCAGATTCTC CGGAAGCGGT AGCGGCACCG ACTACACCTT CACCATCAGC 240  
 AGCCTCCAGC CAGAGGATAT CGCCACCTAC TACTGCCAGC AGAGGAGTAC TTACCCGCTC 300  
 ACGTTCGGCC AAGGGACCAA GCTCGAGATC AAACGGACTA GT 342

15

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs  
 20 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

30

GACATCCAGA TGACCCAGAG CCCAAGCAGC CTGAGCGCTA GCGTGGGTGA CAGAGTGACC 60  
 ATCACGTGTA GTGCCAGCTC AAGTGTAAC TACATGCACT GGTACCAGCA GAAGCCAGGT 120  
 35 AAGGCTCCAA AGCTGCTGAT CTACAGCACA TCCAACCTGG CTTCTGGTGT GCCAAGCAGA 180  
 TTCTCCGGAA GCGGTAGCGG CACCGACTAC ACCTTCACCA TCAGCAGCCT CCAGCCAGAG 240

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GATATCGCCA CCTACTACTG CCAGCAGAGG AGTACTTACC CGCTCACGTT CGGCCAAGGG 300

ACCAAGCTCG AGATCAAACG G 321

## 5 (2) INFORMATION FOR SEQ ID NO: 50:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids

(B) TYPE: amino acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

20 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Tyr Met  
20 25 3025 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 4530 Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
65 70 75 8035 Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Thr Tyr Pro Leu Thr  
85 90 95



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Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg

100

105

(2) INFORMATION FOR SEQ ID NO: 51:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 705 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

15

ATGGATTTTC AAGTCAGAT TTCAGCTTC CTGCTAATCA GTGCTTCAGT CATAATGTCC 60

CGCGGCGACA TCCAGATGAC CCAGAGCCCA AGCAGCCTGA GCGCTAGCGT GGGTGACAGA 120

20 GTGACCATCA CGTGTAGTGC CAGCTCAAGT GTAACCTACA TGCCTGGTA CCAGCAGAAG 180

CCAGGTAAGG CTCAAAGCT GCTGATCTAC AGCACATCCA ACCTGGCTTC TGGTGTGCCA 240

AGCAGATTCT CCGGAAGCGG TAGCGGCACC GACTACACCT TCACCATCAG CAGCCTCCAG 300

25

CCAGAGGATA TGCCACCTA CTA CTGCCAG CAGAGGAGTA CTTACCGCT CACGTTGGGC 360

CAAGGGACCA AGCTCGAGAT CAAACGGACT GTGGCTGCAC CATCTGTCTT CATCTTCCCG 420

30 CCATCTGATG AGCAGTTGAA ATCTGGAACCT GCCTCTGTTG TGTGCCTGCT GAATAACTTC 480

TATCCAGAG AGGCCAAAGT ACAGTGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC 540

CAGGAGAGTG TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCCTG 600

35

ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT CACCCATCAG 660

GGCCTGAGTT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGT 705

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## (2) INFORMATION FOR SEQ ID NO: 52:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 235 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: protein

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser  
 1 5 10 15

20 Val Ile Met Ser Arg Gly Asp Ile Gln Met Thr Gln Ser Pro Ser Ser  
 20 25 30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser  
 35 40 45

25 Ser Ser Val Thr Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala  
 50 55 60

Pro Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro  
 30 65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile  
 85 90 95

35 Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg  
 100 105 110

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Ser Thr Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
 115 120 125

5 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu  
 130 135 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe  
 145 150 155 160

10 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln  
 165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser  
 180 185 190

15 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu  
 195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser  
 20 210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 225 230 235

25 (2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 385 base pairs  
 (B) TYPE: nucleic acid  
 30 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GAAGCTTGGA ATTCAGTGTG AGGTGCAGCT GCAGCAGAGC GGTCCAGGTC TCGTACGGCC 60

5 TAGCCAGACC CTGAGCCTCA CGTGACCGC ATCTGGCTTC AACATTAAGG ACAATTACAT 120

GCACTGGGTG AGACAGCCAC CTGGACGAGG CCTTGAGTGG ATTGGATGGA TTGACCCTGA 180

GAATGGTGAC ACTGAGTACG CACCTAAGTT TCGCGGCCGC GTGACAATGC TGGCAGACAC 240

10 TAGTAAGAAC CAGTTCAGCC TGAGACTCAG CAGCGTGACA GCGCCGACA CCGCGGTCTA 300

TTATTGTAC GTCCTGATAT ACGCCGGGTA TCTGGCAATG GACTACTGGG GCCAAGGGAC 360

15 CCTCGTCACC GTGAGCTCGA CTAGT 385

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 360 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

30 GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60

ACGTGCACCG CATCTGGCTT CAACATTAAG GACAATTACA TGCACTGGGT GAGACAGCCA 120

CCTGGACGAG GCCTTGAGTG GATTGGATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180

35 GCACCTAAGT TTCGCGGCCG CGTGACAATG CTGGCAGACA CTAGTAAGAA CCAGTTCAGC 240

CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCGCGGTCT ATTATTGTCA CGTCCTGATA 300

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TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360

## 5 (2) INFORMATION FOR SEQ ID NO: 55:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

(B) TYPE: amino acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

20 Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln  
1 5 10 15Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
20 25 3025 Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
35 40 4530 Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
50 55 60Arg Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser  
65 70 75 8035 Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

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His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln  
100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
5 115 120

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 765 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

ATGAAGTTGT GGCTGAACTG GATTTCCTT GTAACACTTT TAAATGGAAT TCAGTGTGAG 60  
GTGCAGCTGC AGCAGAGCGG TCCAGGTCTC GTACGGCCTA GCCAGACCCT GAGCCTCACG 120  
25 TGCACCGCAT CTGGCTTCAA CATTAAAGGAC AATTACATGC ACTGGGTGAG ACAGCCACCT 180  
GGACGAGGCC TTGAGTGGAT TGGATGGATT GACCCTGAGA ATGGTGACAC TGAGTACGCA 240  
30 CCTAAGTTTC GCGGCCGCGT GACAATGCTG GCAGACACTA GTAAGAACCA GTTCAGCCTG 300  
AGACTCAGCA GCGTGACAGC CGCCGACACC GCGGTCTATT ATTGTACAGT CCTGATATAC 360  
GCCGGGTATC TGGCAATGGA CTA CTGGGGC CAAGGGACCC TCGTCACCGT GAGCTCGGCT 420  
35 AGCACCAAGG GACCATCGGT CTTCCCCCTG GCCCCCTGCT CCAGGAGCAC CTCCGAGAGC 480  
ACAGCCGCCC TGGGCTGCCT GGTCAAGGAC TACTTCCCCG AACCGGTGAC GGTGTCGTGG 540

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AACTCAGGCG CTCTGACCAG CGGCGTGAC ACCTTCCCGG CTGTCCTACA GTCCTCAGGA 600

CTCTACTCCC TCAGCAGCGT CGTGACGGTG CCCTCCAGCA ACTTCGGCAC CCAGACCTAC 660

5

ACCTGCAACG TAGATCACAA GCCCAGCAAC ACCAAGGTGG ACAAGACAGT TGAGCGCAAA 720

TGTTGTGTCG AGTGCCCAAC GTGCCCGGCG CCACCTGTGG CCGGC 765

## 10 (2) INFORMATION FOR SEQ ID NO: 57:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 255 amino acids

(B) TYPE: amino acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

25

Met Lys Leu Trp Leu Asn Trp Ile Phe Leu Val Thr Leu Leu Asn Gly

1 5 10 15

Ile Gln Cys Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg

20 25 30

30

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile

35 40 45

Lys Asp Asn Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu

35

50 55 60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala

65 70 75 80

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Pro Lys Phe Arg Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn

85

90

95

5 Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val

100

105

110

Tyr Tyr Cys His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr

115

120

125

10

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly

130

135

140

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser

15

145

150

155

160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val

165

170

175

20

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

180

185

190

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val

195

200

205

25

Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val

210

215

220

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys

30

225

230

235

240

Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly

245

250

255

35 (2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs



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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GGCGACATCC AGCTGACCCA GAGCCCAAGC AGCCTGAGCG

40

10

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

25

CTAGCGCTCA GGCTGCTTGG GCTCTGGGTC AGCTGGATGT CGCCGC

46

(2) INFORMATION FOR SEQ ID NO: 60:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 321 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

- 132 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GACATCCAGC TGACCCAGAG CCCAAGCAGC CTGAGCGCTA GCGTGGGTGA CAGAGTGACC 60  
 5 ATCACGTGTA GTGCCAGCTC AAGTGTAAC TACATGCACT GGTACCAGCA GAAGCCAGGT 120  
 AAGGCTCCAA AGCTGCTGAT CTACAGCACA TCCAACCTGG CTTCTGGTGT GCCAAGCAGA 180  
 TTCTCCGAA GCGGTAGCGG CACCGACTAC ACCTTCACCA TCAGCAGCCT CCAGCCAGAG 240  
 10 GATATGCCA CCTACTACTG CCAGCAGAGG AGTACTTACC CGCTCACGTT CGGCCAAGGG 300  
 ACCAAGCTCG AGATCAAACG G 321

15 (2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids  
 (B) TYPE: amino acid  
 20 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

30 Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Tyr Met  
 20 25 30  
 35 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45

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Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
 5 65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Thr Tyr Pro Leu Thr  
 85 90 95

10 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105

(2) INFORMATION FOR SEQ ID NO: 62:

- 15 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

GGCCAGATCG TGCTGACCCA GAGCCCAAGC AGCCTGAGCG

40

30 (2) INFORMATION FOR SEQ ID NO: 63:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 46 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- 35

(ii) MOLECULE TYPE: other nucleic acid

- 134 -

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

CTAGCGCTCA GGCTGCTTGG GCTCTGGGTC AGCACGATCT GGCCGC

46

(2) INFORMATION FOR SEQ ID NO: 64:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

CAGATCGTGC TGACCCAGAG CCCAAGCAGC CTGAGCGCTA GCGTGGGTGA CAGAGTGACC

60

25

ATCACGTGTA GTGCCAGCTC AAGTGTA ACT TACATGCACT GGTACCAGCA GAAGCCAGGT

120

AAGGCTCCAA AGCTGCTGAT CTACAGCACA TCCAACCTGG CTTCTGGTGT GCCAAGCAGA

180

30

TTCTCCGGAA GCGGTAGCGG CACCGACTAC ACCTTCACCA TCAGCAGCCT CCAGCCAGAG

240

GATATCGCCA CCTACTACTG CCAGCAGAGG AGTACTTACC CGCTCACGTT CGGCCAAGGG

300

ACCAAGCTCG AGATCAAACG G

321

35

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

- 135 -

(A) LENGTH: 107 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

Gln Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1                      5                      10                      15

15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Tyr Met  
                     20                      25                      30

20

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
                     35                      40                      45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
                     50                      55                      60

25

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
                     65                      70                      75                      80

30

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Thr Tyr Pro Leu Thr  
                     85                      90                      95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
                     100                      105

35 (2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

- 136 -

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: other nucleic acid

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

CGTATTAGTC ATCGCTATTA CC

22

(2) INFORMATION FOR SEQ ID NO: 67:

15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

GTTGGATGTG CTGTAGATCC ACAGCTTTGG AGCCTTACC

39

(2) INFORMATION FOR SEQ ID NO: 68:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

- 137 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

5

TCCGTTTGAT CTCGAGCTTG G

21

(2) INFORMATION FOR SEQ ID NO: 69:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

GGTAAGGCTC CAAAGCTGTG GATCTACAGC ACATCCAAC

39

25 (2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

GACATCCAGA TGACCCAGAG CCCAAGCAGC CTGAGCGCTA GCGTGGGTGA CAGAGTGACC 60  
 5 ATCACGTGTA GTGCCAGCTC AAGTGTAAC TACATGCACT GGTACCAGCA GAAGCCAGGT 120  
 AAGGCTCCAA AGCTGTGGAT CTACAGCACA TCCAACCTGG CTTCTGGTGT GCCAAGCAGA 180  
 TTCTCCGGAA GCGGTAGCGG CACCGACTAC ACCTTCACCA TCAGCAGCCT CCAGCCAGAG 240  
 10 GATATCGCCA CCTACTACTG CCAGCAGAGG AGTACTTACC CGCTCACGTT CGGCCAAGGG 300  
 ACCAAGCTCG AGATCAAACG G 321

15 (2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids  
 (B) TYPE: amino acid  
 20 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

30 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Thr Tyr Met  
 20 25 30  
 35 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Trp Ile Tyr  
 35 40 45



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Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
 5 65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Thr Tyr Pro Leu Thr  
 85 90 95

10 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg  
 100 105

(2) INFORMATION FOR SEQ ID NO: 72:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 64 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: other nucleic acid

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

CCTTGAGTGG ATTGCATGGA TTGACCCTGA GAATGGTGAC ACTGAGTACG CACCTAAGTT 60

30 TCGC 64

(2) INFORMATION FOR SEQ ID NO: 73:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 68 base pairs  
 (B) TYPE: nucleic acid

- 140 -

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

10

GGCCGCGAAA CTTAGGTGCG TACTCAGTGT CACCATTCTC AGGGTCAATC CATGCAATCC 60

ACTCAAGG 68

15 (2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 base pairs

(B) TYPE: nucleic acid

20

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

30 GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60

ACGTGCACCG CATCTGGCTT CAACATTAAG GACAATTACA TGCACTGGGT GAGACAGCCA 120

CCTGGACGAG GCCTTGAGTG GATTGCATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180

35

GCACCTAAGT TTCGCGGCCG CGTGACAATG CTGGCAGACA CTAGTAAGAA CCAGTTCAGC 240

CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCCGGGTCT ATTATTGTCA CGTCCTGATA 300

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TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360

(2) INFORMATION FOR SEQ ID NO: 75:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

10

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln

20            1                 5                      10                          15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
20 25 30

25 Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
35 40 45

Ala Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
50 55 60

30

Arg Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser  
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln  
100 105 110

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Gly Thr Leu Val Thr Val Ser Ser

115

120

## 5 (2) INFORMATION FOR SEQ ID NO: 76:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 80 base pairs

(B) TYPE: nucleic acid

10

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

20 GGCCGCGTGA CAATGCTGGC AGACTCAAGT AAGAACCAGG CCAGCCTGAG ACTCAGCAGC 60

GTGACAGCCG CCGACACCGC 80

## (2) INFORMATION FOR SEQ ID NO: 77:

25

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 74 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

35

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

GGTGTGGGCG GCTGTCACGC TGCTGAGTCT CAGGCTGGCC TGGTTCTTAC TTGAGTCTGC 60  
5 CAGCATTGTC ACGC 74

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH: 360 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

25 GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60  
ACGTGCACCG CATCTGGCTT CAACATTAA GACAATTACA TGCACTGGGT GAGACAGCCA 120  
CCTGGACGAG GCCTTGAGTG GATTGGATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180  
30 GCACCTAAGT TTCGCGGCCG CGTGACAATG CTGGCAGACT CAAGTAAGAA CCAGGCCAGC 240  
CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCGCGGTCT ATTATTGTCA CGTCCTGATA 300  
35 TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360

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## (2) INFORMATION FOR SEQ ID NO: 79:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln

1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn

20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile

35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe

50 55 60

Arg Gly Arg Val Thr Met Leu Ala Asp Ser Ser Lys Asn Gln Ala Ser

65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys

85 90 95

His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln

100 105 110

Gly Thr Leu Val Thr Val Ser Ser

115 120

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## (2) INFORMATION FOR SEQ ID NO: 80:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 360 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: other nucleic acid

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60  
ACGTGCACCG CATCTGGCTT CAACATTAAAG GACAATTACA TGCCTGGGT GAGACAGCCA 120  
20 CCTGGACGAG GCCTTGAGTG GATTGCATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180  
GCACCTAAGT TTCGCGGCCG CGTGACAATG CTGGCAGACT CAAGTAAGAA CCAGGCCAGC 240  
25 CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCGCGGTCT ATTATTGTCA CGTCCTGATA 300  
TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360

## 30 (2) INFORMATION FOR SEQ ID NO: 81:

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 120 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln  
 5      1                      5                      10                      15  
 Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
 20                      25                      30  
 Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
 35                      40                      45  
 Ala Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
 50                      55                      60  
 Arg Gly Arg Val Thr Met Leu Ala Asp Ser Ser Lys Asn Gln Ala Ser  
 65                      70                      75                      80  
 Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys  
 85                      90                      95  
 His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln  
 100                      105                      110  
 Gly Thr Leu Val Thr Val Ser Ser  
 115                      120

(2) INFORMATION FOR SEQ ID NO: 82:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 80 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

5

GGCCGCGCCA CAATGCTGGC AGACACTAGT AAGAACCAGT TCAGCCTGAG ACTCAGCAGC 60

GTGACAGCCG CCGACACCGC 80

10 (2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 74 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

25 GGTGTCGGCG GCTGTCACGC TGCTGAGTCT CAGGCTGAAC TGGTTCTTAC TAGTGTCTGC 60

CAGCATTGTG GCGC 74

(2) INFORMATION FOR SEQ ID NO: 84:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

5 GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60  
 ACGTGCACCG CATCTGGCTT CAACATTAAG GACAATTACA TGCACTGGGT GAGACAGCCA 120  
 CCTGGACGAG GCCTTGAGTG GATTGGATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180  
 10 GCACCTAAGT TTCGCGGCCG CGCCACAATG CTGGCAGACA CTAGTAAGAA CCAGTTCAGC 240  
 CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCGCGGTCT ATTATTGTCA CGTCCTGATA 300  
 TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360  
 15

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 120 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln  
 1 5 10 15  
 Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
 20 25 30

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Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
 35 40 45

5 Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
 50 55 60

Arg Gly Arg Ala Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser  
 65 70 75 80

10 Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

15 His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln  
 100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
 115 120

(2) INFORMATION FOR SEQ ID NO: 86:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 80 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

GGCCGCGCCA CAATGCTGGC AGACTCAAGT AAGAACCAGG CCAGCCTGAG ACTCAGCAGC 60  
 35  
 GTGACAGCCG CCGACACCGC 80

- 150 -

## (2) INFORMATION FOR SEQ ID NO: 87:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 74 base pairs

5 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

10

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

15

GGTGTGGCG GCTGTCACGC TGCTGAGTCT CAGGCTGGCC TGGTTCTTAC TTGAGTCTGC 60

CAGCATTGTG GCGC 74

## 20 (2) INFORMATION FOR SEQ ID NO: 88:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 base pairs

25 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

35 GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60

ACGTGCACCG CATCTGGCTT CAACATTAAG GACAATTACA TGCACTGGGT GAGACAGCCA 120

- 151 -

CCTGGACGAG GCCTTGAGTG GATTGGATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180

GCACCTAAGT TTCGCGGCCG CGCCACAATG CTGGCAGACT CAAGTAAGAA CCAGGCCAGC 240

5 CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCGCGGTCT ATTATTGTCA CGTCCTGATA 300

TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360

## 10 (2) INFORMATION FOR SEQ ID NO: 89:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 120 amino acids
- (B) TYPE: amino acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

25 Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln  
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
20 25 30

30 Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
35 50 55 60

Arg Gly Arg Ala Thr Met Leu Ala Asp Ser Ser Lys Asn Gln Ala Ser  
65 70 75 80

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Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys

85

90

95

5 His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln

100

105

110

Gly Thr Leu Val Thr Val Ser Ser

115

120

10

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 360 base pairs

15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

25

GAGGTGCAGC TGCAGCAGAG CGGTCCAGGT CTCGTACGGC CTAGCCAGAC CCTGAGCCTC 60

ACGTGCACCG CATCTGGCTT CAACATTAAG GACAATTACA TGCACTGGGT GAGACAGCCA 120

30 CCTGGACGAG GCCTTGAGTG GATTGCATGG ATTGACCCTG AGAATGGTGA CACTGAGTAC 180

GCACCTAAGT TTCGCGGCCG CGCCACAATG CTGGCAGACT CAAGTAAGAA CCAGGCCAGC 240

CTGAGACTCA GCAGCGTGAC AGCCGCCGAC ACCGCGGTCT ATTATTGTCA CGTCCTGATA 300

35

TACGCCGGGT ATCTGGCAAT GGACTACTGG GGCCAAGGGA CCCTCGTCAC CGTGAGCTCG 360

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## (2) INFORMATION FOR SEQ ID NO: 91:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 amino acids

5 (B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln  
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn  
20 25 30

Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile  
35 40 45

Ala Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe  
25 50 55 60

Arg Gly Arg Ala Thr Met Leu Ala Asp Ser Ser Lys Asn Gln Ala Ser  
65 70 75 80

Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln  
35 100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
115 120

- 154 -

## (2) INFORMATION FOR SEQ ID NO: 92:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 780 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- 10 (ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

15

ATGAAGTTGT GGCTGAACTG GATTTTCCTT GTAACACTTT TAAATGGAAT TCAGTGTGAG 60

GTGCAGCTGC AGCAGAGCGG TCCAGGTCTC GTACGGCCTA GCCAGACCCT GAGCCTCAGC 120

20 TGCACCGCAT CTGGCTTCAA CATTAAGGAC AATTACATGC ACTGGGTGAG ACAGCCACCT 180

GGACGAGGCC TTGAGTGGAT TGGATGGATT GACCCTGAGA ATGGTGACAC TGAGTACGCA 240

CCTAAGTTTC GCGGCCGCGT GACAATGCTG GCAGACACTA GTAAGAACCA GTTCAGCCTG 300

25

AGACTCAGCA GCGTGACAGC CGCCGACACC GCGGTCTATT ATTGTCACGT CCTGATATAC 360

GCCGGGTATC TGGCAATGGA CTACTGGGGC CAAGGGACCC TCGTCACCGT GAGCTCGGCC 420

30 TCCACCAAGG GCCCATCGGT CTTCCCCCTG GCACCTCCT CCAAGAGCAC CTCTGGGGGC 480

ACAGCGGCCC TGGGCTGCCT GGTCAAGGAC TACTTCCCCG AACCGGTGAC GGTGTGCTGG 540

AACTCAGGCG CCCTGACCAG CGGCGTGAC ACCTTCCCGG CTGTCCTACA GTCCTCAGGA 600

35

CTCTACTCCC TCAGCAGCGT GGTGACTGTG CCCTCCAGCA GCTTGGGCAC CCAGACCTAC 660

ATCTGCAACG TGAATCACAA CCCCAGCAAC ACCAAGGTCG ACAAGAAAGT TGAGCCCAAA 720



- 155 -

TCTTGTGACA AGACGCACAC GTGCCCGCCG TGCCCGGCTC CGGAACTGCT GGGTGGCCCG 780

## 5 (2) INFORMATION FOR SEQ ID NO: 93:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 260 amino acids  
 (B) TYPE: amino acid  
 10 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

20 Met Lys Leu Trp Leu Asn Trp Ile Phe Leu Val Thr Leu Leu Asn Gly  
 1 5 10 15

Ile Gln Cys Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg  
 20 25 30

25

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile  
 35 40 45

30

Lys Asp Asn Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu  
 50 55 60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala  
 65 70 75 80

35

Pro Lys Phe Arg Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn  
 85 90 95

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Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val  
 100 105 110

5 Tyr Tyr Cys His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr  
 115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly  
 130 135 140

10 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly  
 145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val  
 165 170 175

15 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe  
 180 185 190

20 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val  
 195 200 205

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val  
 210 215 220

25 Asn His Asn Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys  
 225 230 235 240

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
 245 250 255

30 Leu Gly Gly Pro  
 260

(2) INFORMATION FOR SEQ ID NO: 94:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 918 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

10

ATGAAGTTGT GGCTGAACTG GATTTTCCTT GTAACACTTT TAAATGGAAT TCAGTGTGAG 60

GTGCAGCTGC AGCAGAGCGG TCCAGGTCTC GTACGGCCTA GCCAGACCCT GAGCCTCAGC 120

15 TGCACCGCAT CTGGCTTCAA CATTAAAGGAC AATTACATGC ACTGGGTGAG ACAGCCACCT 180

GGACGAGGCC TTGAGTGGAT TGGATGGATT GACCCTGAGA ATGGTGACAC TGAGTACGCA 240

CCTAAGTTTC GCGGCCGCGT GACAATGCTG GCAGACACTA GTAAGAACCA GTTCAGCCTG 300

20

AGACTCAGCA GCGTGACAGC CGCCGACACC GCGGTCTATT ATTGTCACGT CCTGATATAC 360

GCCGGGTATC TGGCAATGGA CTACTGGGGC CAAGGGACCC TCGTCACCGT GAGCTCGGCT 420

25 AGCACCAAGG GCCCATCGGT CTCCCCCTG GCGCCCTGCT CCAGGAGCAC CTCTGGGGGC 480

ACAGCGGCCC TGGGCTGCCT GGTC AAGGAC TACTTCCCCG AACCGGTGAC GGTGTCGTGG 540

AACTCAGGCG CCCTGACCAG CGGCGTGAC ACCTTCCCGG CTGTCCTACA GTCCTCAGGA 600

30

CTCTACTCCC TCAGCAGCGT GGTGACCGTG CCCTCCAGCA GCTTGGGCAC CCAGACCTAC 660

ACCTGCAACG TGAATCACAA GCCCAGCAAC ACCAAGGTGG ACAAGAGAGT GGAGCTGAAA 720

35 ACCCCACTCG GTGACACAAC TCACAGTGC CTTAGGTGTC CTGAACCTAA ATCTTGTGAC 780

ACACCTCCCC CGTGCCACG GTGCCAGAG CCCAAATCTT GCGACACGCC CCCACCGTGT 840

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CCCAGATGTC CTGAACCAAA GAGCTGTGAC ACTCCACCGC CCTGCCCGAG GTGCCCAAGCA 900

CCTGAACTCC TGGGAGGG 918

5 (2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 amino acids  
 (B) TYPE: amino acid  
 10 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

20 Met Lys Leu Trp Leu Asn Trp Ile Phe Leu Val Thr Leu Leu Asn Gly  
 1 5 10 15

Ile Gln Cys Glu Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Arg  
 20 25 30

25

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile  
 35 40 45

30

Lys Asp Asn Tyr Met His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu  
 50 55 60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala  
 65 70 75 80

35

Pro Lys Phe Arg Gly Arg Val Thr Met Leu Ala Asp Thr Ser Lys Asn  
 85 90 95

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Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val  
 100 105 110

5 Tyr Tyr Cys His Val Leu Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr  
 115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly  
 130 135 140

10 Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Gly Gly  
 145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val  
 15 165 170 175

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe  
 180 185 190

20 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val  
 195 200 205

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Thr Cys Asn Val  
 210 215 220

25 Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Leu Lys  
 225 230 235 240

Thr Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys Pro Glu Pro  
 30 245 250 255

Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu Pro Lys  
 260 265 270

35 Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Glu Pro Lys Ser  
 275 280 285

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Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro Ala Pro Glu Leu Leu  
290 295 300

5 Gly Gly  
305

(2) INFORMATION FOR SEQ ID NO: 96:

- (i) SEQUENCE CHARACTERISTICS:
- 10 (A) LENGTH: 705 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 15 (ii) MOLECULE TYPE: other nucleic acid

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

ATGGATTTTC AAGTGCAGAT TTTCAGCTTC CTGCTAATCA GTGCTTCAGT CATAATGTCC 60

CGCGGCCAGA TCGTGCTGAC CCAGAGCCCA AGCAGCCTGA GCGCTAGCGT GGGTGACAGA 120

25 GTGACCATCA CGTGTAGTGC CAGCTCAAGT GTAACCTACA TGCACTGGTA CCAGCAGAAG 180

CCAGGTAAGG CTCCAAAGCT GCTGATCTAC AGCACATCCA ACCTGGCTTC TGGTGTGCCA 240

30 AGCAGATTCT CCGGAAGCGG TAGCGGCACC GACTACACCT TCACCATCAG CAGCCTCCAG 300

CCAGAGGATA TCGCCACCTA CTACTGCCAG CAGAGGAGTA CTTACCCGCT CACGTTCCGG 360

CAAGGGACCA AGCTCGAGAT CAAACGGACT GTGGCTGCAC CATCTGTCTT CATCTTCCCG 420

35 CCATCTGATG AGCAGTTGAA ATCTGGAAGT GCCTCTGTTG TGTGCCTGCT GAATAACTTC 480

TATCCCAGAG AGGCCAAAGT ACAGTGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC 540

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CAGGAGAGTG TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCCCTG 600

ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CTTGCGAAGT CACCCATCAG 660

5

GGCCTGAGTT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGT 705

(2) INFORMATION FOR SEQ ID NO: 97:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 235 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser  
1 5 10 15

25

Val Ile Met Ser Arg Gly Gln Ile Val Leu Thr Gln Ser Pro Ser Ser  
20 25 30

30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser  
35 40 45

Ser Ser Val Thr Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala  
50 55 60

35

Pro Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro  
65 70 75 80

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Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile  
 85 90 95

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg  
 5 100 105 110

Ser Thr Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
 115 120 125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu  
 10 130 135 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe  
 145 150 155 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln  
 15 165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser  
 20 180 185 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu  
 195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser  
 25 210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 225 230 235

30

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 705 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35



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(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

ATGGATTTTC AAGTGCAGAT TTTCAGCTTC CTGCTAATCA GTGCTTCAGT CATAATGTCC 60

10 CGCGGCGACA TCCAGATGAC CCAGAGCCCA AGCAGCCTGA GCGCTAGCGT GGGTGACAGA 120

GTGACCATCA CGTGTACTGC CAGCTCAAGT GTAACCTTACA TGCACTGGTA CCAGCAGAAG 180

CCAGGTAAGG CTCCAAAGCT GTGGATCTAC AGCACATCCA ACCTGGCTTC TGGTGTGCCA 240

15 AGCAGATTCT CCGGAAGCGG TAGCGGCACC GACTACACCT TCACCATCAG CAGCCTCCAG 300

CCAGAGGATA TCGCCACCTA CTA CTGCCAG CAGAGGAGTA CTTACCCGCT CACGTTCCGGC 360

20 CAAGGGACCA AGCTCGAGAT CAAACGGA CT GTGGCTGCAC CATCTGTCTT CATCTTCCCG 420

CCATCTGATG AGCAGTTGAA ATCTGGAACT GCCTCTGTTG TGTGCCTGCT GAATAACTTC 480

TATCCCAGAG AGGCCAAAGT ACAGTGGAAG GTGGATAACG CCCTCCAATC GGGTAACTCC 540

25 CAGGAGAGTG TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG CAGCACCTG 600

ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT CACCCATCAG 660

30 GGCCTGAGTT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGT 705

(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 235 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser  
 10      1                      5                      10                      15

Val Ile Met Ser Arg Gly Asp Ile Gln Met Thr Gln Ser Pro Ser Ser  
                     20                      25                      30

15      Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser  
                     35                      40                      45

Ser Ser Val Thr Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala  
                     50                      55                      60

20      Pro Lys Leu Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro  
                     65                      70                      75                      80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile  
 25                      85                      90                      95

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Arg  
                     100                      105                      110

30      Ser Thr Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys  
                     115                      120                      125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu  
                     130                      135                      140

35      Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe  
                     145                      150                      155                      160

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Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln  
165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser  
5 180 185 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu  
195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser  
10 210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
15 225 230 235

(2) INFORMATION FOR SEQ ID NO: 100:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 54 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- 25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

30

CCCAGCACCT GAACTCCTGG GAGGAGCAAC AGGACACAGT TATGAGAAGT ACAA

54

(2) INFORMATION FOR SEQ ID NO: 101:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 35

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

10 GGGGGTCTAG ATTATTAGTA CAGGTGTTCC AGGACGTAGC TGGCAACATA

50

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 46 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

25

GGGGGAGCTC GGCTAGCACC AAGGGCCCAT CGGTCTTCCC CCTGGC

46

(2) INFORMATION FOR SEQ ID NO: 103:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 55 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

TTGTACTTCT CATAACTGTG TCCTGTTGCT CCTCCCAGGA GTTCAGGTGC TGGGC

55

5 (2) INFORMATION FOR SEQ ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

20 GCCTGTGCTC AATATTGATG G

21

(2) INFORMATION FOR SEQ ID NO: 105:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: other nucleic acid

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

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GGAGAAAGCC ATATCTGCCT G

21

(2) INFORMATION FOR SEQ ID NO: 106:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

TCGCTATTAC CATGGTGATG CGGTTTTGGC

30

20 (2) INFORMATION FOR SEQ ID NO: 107:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

25

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

GGCTGGATTG TCAGTGGCGA CTT

23

35

(2) INFORMATION FOR SEQ ID NO: 108:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

CACAAACAGAG GCAGTTCC

18

(2) INFORMATION FOR SEQ ID NO: 109:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

CACCTTCACC ATCAGCAGCC

20

(2) INFORMATION FOR SEQ ID NO: 110:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

10 GGACCTGCTG CAGAGTCTG

19

(2) INFORMATION FOR SEQ ID NO: 111:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

GGCTGCAGGA ATTCTTATTA TAGACGAACC CGGCTATCAA ACTGAGC

47

(2) INFORMATION FOR SEQ ID NO: 112:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1870 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

	AAGCTTGCCG CCACCATGAA GTTGTGGCTG AACTGGATT TCCTTGTAAC ACTTTTAAAT	60
5	GGAATTCAGT GTGAGGTGCA GCTGCAGCAG AGCGGTCCAG GTCTCGTACG GCCTAGCCAG	120
	ACCCTGAGCC TCACGTGCAC CGCATCTGGC TTCAACATTA AGGACAATTA CATGCACTGG	180
	GTGAGACAGC CACCTGGACG AGGCCTTGAG TGGATTGGAT GGATTGACCC TGAGAATGGT	240
10	GAACTGAGT ACGCACCTAA GTTTCGCGGC CGCGTGACAA TGCTGGCAGA CACTAGTAAG	300
	AACCAATTCA GCCTGAGACT CAGCAGCGTG ACAGCCGCCG ACACCGCGGT CTATTATTGT	360
15	CACGTCTGA TATACGCCGG GTATCTGGCA ATGGACTACT GGGGCCAAGG GACCCTCGTC	420
	ACCGTGAGCT CGGCTAGCAC CAAGGGCCCA TCGGTCTTCC CCCTGGCGCC CTGCTCCAGG	480
	AGCACCTCTG GGGGCACAGC GGCCCTGGGC TGCTGGTCA AGGACTACTT CCCGAACCG	540
20	GTGACGGTGT CGTGGAATC AGGCGCCCTG ACCAGCGGCG TGCACACCTT CCCGGCTGTC	600
	CTACAGTCCT CAGGACTCTA CTCCTCAGC AGCGTGGTGA CCGTGCCCTC CAGCAGCTTG	660
25	GGCAGCCAGA CCTACACCTG CAACGTGAAT CACAAGCCCA GCAACACCAA GGTGGACAAG	720
	AGAGTGGAGC TGAAAACCCC ACTCGGTGAC ACAACTCACA CGTGCCCTAG GTGTCCTGAA	780
	CCTAAATCTT GTGACACACC TCCCCCGTGC CCACGGTGCC CAGAGCCCAA ATCTTGCGAC	840
30	ACGCCCCCAC CGTGTECCAG ATGTCTTGAA CCAAAGAGCT GTGACACTCC ACCGCCCTGC	900
	CCGAGGTGCC CAGCACCTGA ACTCCTGGGA GGAGCAACAG GACACAGTTA TGAGAAGTAC	960
35	AACAAGTGGG AAACGATAGA GGCTTGGAAT CAACAAGTCG CCACTGAGAA TCCAGCCCTC	1020
	ATCTCTCGCA GTGTTATCGG AACCACATTT GAGGGACGCG CTATTTACCT CCTGAAGGTT	1080

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GGCAAAGCTG GACAAATAA GCCTGCCATT TTCATGGACT GTGTTTCCA TGCCAGAGAG 1140  
 TGGATTTCCTC CTGCATTCTG CCAGTGGTTT GTAAGAGAGG CTGTTCGTAC CTATGGACGT 1200  
 5 GAGATCCAAG TGACAGAGCT TCTCGACAAG TTAGACTTTT ATGTCCTGCC TGTGCTCAAT 1260  
 ATTGATGGCT ACATCTACAC CTGGACCAAG AGCCGATTTT GGAGAAAGAC TCGCTCCACC 1320  
 CATACTGGAT CTAGCTGCAT TGGCACAGAC CCCAACAGAA ATTTTGATGC TGGTTGGTGT 1380  
 10 GAAATTGGAG CCTCTCGAAA CCCCTGTGAT GAAACTTACT GTGGACCTGC CGCAGAGTCT 1440  
 GAAAAGGAGA CCAAGGCCCT GGCTGATTTC ATCCGCAACA AACTCTCTTC CATCAAGGCA 1500  
 15 TATCTGACAA TCCACTCGTA CTCCCAAATG ATGATCTACC CTTACTCATA TGCTTACAAA 1560  
 CTCGGTGAGA ACAATGCTGA GTTGAATGCC CTGGCTAAAG CTACTGTGAA AGAACTTGCC 1620  
 TCACTGCACG GCACCAAGTA CACATATGGC CCGGGAGCTA CAACAATCTA TCCTTCTGCT 1680  
 20 GGGACTTCTA AAGACTGGGC TTATGACCAA GGAATCAGAT ATTCCTTCAC CTTTGAAGTT 1740  
 CGAGATACAG GCAGATATGG CTTTCTCCTT CCAGAATCCC AGATCCGGGC TACCTGCGAG 1800  
 25 GAGACCTTCC TGGCAATCAA GTATGTTGCC AGCTACGTCC TGGAACACCT GTACTAATAA 1860  
 TCTAGAGAGA 1870

(2) INFORMATION FOR SEQ ID NO: 113:

30

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 613 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

35

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

	Met	Lys	Leu	Trp	Leu	Asn	Trp	Ile	Phe	Leu	Val	Thr	Leu	Leu	Asn	Gly	
	1				5					10					15		
5																	
	Ile	Gln	Cys	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Gly	Leu	Val	Arg	
				20					25					30			
	Pro	Ser	Gln	Thr	Leu	Ser	Leu	Thr	Cys	Thr	Ala	Ser	Gly	Phe	Asn	Ile	
10				35				40						45			
	Lys	Asp	Asn	Tyr	Met	His	Trp	Val	Arg	Gln	Pro	Pro	Gly	Arg	Gly	Leu	
		50					55						60				
15																	
	Glu	Trp	Ile	Gly	Trp	Ile	Asp	Pro	Glu	Asn	Gly	Asp	Thr	Glu	Tyr	Ala	
	65				70					75					80		
	Pro	Lys	Phe	Arg	Gly	Arg	Val	Thr	Met	Leu	Ala	Asp	Thr	Ser	Lys	Asn	
				85					90					95			
20																	
	Gln	Phe	Ser	Leu	Arg	Leu	Ser	Ser	Val	Thr	Ala	Ala	Asp	Thr	Ala	Val	
				100					105					110			
	Tyr	Tyr	Cys	His	Val	Leu	Ile	Tyr	Ala	Gly	Tyr	Leu	Ala	Met	Asp	Tyr	
25				115					120					125			
	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	
		130					135						140				
30																	
	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Gly	Gly	
	145					150					155				160		
	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	
				165					170					175			
35																	
	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	
				180					185					190			

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	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	
				195				200					205				
5	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Thr	Cys	Asn	Val	
				210			215					220					
	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Leu	Lys	
	225					230					235				240		
10	Thr	Pro	Leu	Gly	Asp	Thr	Thr	His	Thr	Cys	Pro	Arg	Cys	Pro	Glu	Pro	
					245					250					255		
	Lys	Ser	Cys	Asp	Thr	Pro	Pro	Pro	Cys	Pro	Arg	Cys	Pro	Glu	Pro	Lys	
15				260					265					270			
	Ser	Cys	Asp	Thr	Pro	Pro	Pro	Cys	Pro	Arg	Cys	Pro	Glu	Pro	Lys	Ser	
				275				280					285				
	Cys	Asp	Thr	Pro	Pro	Pro	Cys	Pro	Arg	Cys	Pro	Ala	Pro	Glu	Leu	Leu	
20				290			295					300					
	Gly	Gly	Ala	Thr	Gly	His	Ser	Tyr	Glu	Lys	Tyr	Asn	Lys	Trp	Glu	Thr	
	305				310					315					320		
25	Ile	Glu	Ala	Trp	Thr	Gln	Gln	Val	Ala	Thr	Glu	Asn	Pro	Ala	Leu	Ile	
					325					330					335		
	Ser	Arg	Ser	Val	Ile	Gly	Thr	Thr	Phe	Glu	Gly	Arg	Ala	Ile	Tyr	Leu	
30				340					345					350			
	Leu	Lys	Val	Gly	Lys	Ala	Gly	Gln	Asn	Lys	Pro	Ala	Ile	Phe	Met	Asp	
				355				360					365				
35	Cys	Gly	Phe	His	Ala	Arg	Glu	Trp	Ile	Ser	Pro	Ala	Phe	Cys	Gln	Trp	
				370				375					380				

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	Phe Val Arg Glu Ala Val Arg Thr Tyr Gly Arg Glu Ile Gln Val Thr	
	385	390 395 400
5	Glu Leu Leu Asp Lys Leu Asp Phe Tyr Val Leu Pro Val Leu Asn Ile	
	405	410 415
	Asp Gly Tyr Ile Tyr Thr Trp Thr Lys Ser Arg Phe Trp Arg Lys Thr	
	420	425 430
10	Arg Ser Thr His Thr Gly Ser Ser Cys Ile Gly Thr Asp Pro Asn Arg	
	435	440 445
	Asn Phe Asp Ala Gly Trp Cys Glu Ile Gly Ala Ser Arg Asn Pro Cys	
	450	455 460
15	Asp Glu Thr Tyr Cys Gly Pro Ala Ala Glu Ser Glu Lys Glu Thr Lys	
	465	470 475 480
	Ala Leu Ala Asp Phe Ile Arg Asn Lys Leu Ser Ser Ile Lys Ala Tyr	
20	485	490 495
	Leu Thr Ile His Ser Tyr Ser Gln Met Met Ile Tyr Pro Tyr Ser Tyr	
	500	505 510
25	Ala Tyr Lys Leu Gly Glu Asn Asn Ala Glu Leu Asn Ala Leu Ala Lys	
	515	520 525
	Ala Thr Val Lys Glu Leu Ala Ser Leu His Gly Thr Lys Tyr Thr Tyr	
	530	535 540
30	Gly Pro Gly Ala Thr Thr Ile Tyr Pro Ser Ala Gly Thr Ser Lys Asp	
	545	550 555 560
	Trp Ala Tyr Asp Gln Gly Ile Arg Tyr Ser Phe Thr Phe Glu Leu Arg	
35	565	570 575
	Asp Thr Gly Arg Tyr Gly Phe Leu Leu Pro Glu Ser Gln Ile Arg Ala	
	580	585 590

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Thr Cys Glu Glu Thr Phe Leu Ala Ile Lys Tyr Val Ala Ser Tyr Val  
 595 600 605

5 Leu Glu His Leu Tyr  
 610

(2) INFORMATION FOR SEQ ID NO: 114:

10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 96 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

His His Gly Gly Glu His Phe Glu Gly Glu Lys Val Phe Arg Val Asn  
 1 5 10 15

25 Val Glu Asp Glu Asn His Ile Asn Ile Ile Arg Glu Leu Ala Ser Thr  
 20 25 30

Thr Gln Ile Asp Phe Trp Lys Pro Asp Ser Val Thr Gln Ile Lys Pro  
 35 40 45

30 His Ser Thr Val Asp Phe Arg Val Lys Ala Glu Asp Thr Val Thr Val  
 50 55 60

Glu Asn Val Leu Lys Gln Asn Glu Leu Gln Tyr Lys Val Leu Ile Ser  
 35 65 70 75 80

Asn Leu Arg Asn Val Val Glu Ala Gln Phe Asp Ser Arg Val Arg Leu  
 85 90 95

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## (2) INFORMATION FOR SEQ ID NO: 115:

- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 520 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

10

- (ii) MOLECULE TYPE: other nucleic acid

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

GAGCTCGGCT AGCACCAAGG GCCCATCGGT CTCCCCCTG GCGCCCTGCT CCAGGAGCAC 60

20 CTCTGGGGGC ACAGCGGCCC TGGGCTGCCT GGTCAAGGAC TACTTCCCCG AACCGGTGAC 120

GGTGTCTGG AACTCAGGCG CCTGACCAG CGGCGTGAC ACCTTCCCGG CTGTCCTACA 180

GTCCTCAGGA CTCTACTCCC TCAGCAGCGT GGTGACCGTG CCCTCCAGCA GCTTGGGCAC 240

25 CCAGACCTAC ACCTGCAACG TGAATCACAA GCCCAGCAAC ACCAAGGTGG ACAAGAGAGT 300

GGAGCTGAAA ACCCCACTCG GTGACACAAC TCACACGTGC CCTAGGTGTC CTGAACCTAA 360

30 ATCTTGTGAC ACACCTCCCC CGTGCCCACG GTGCCCAGAG CCCAAATCTT GCGACACGCC 420

CCCACCGTGT CCCAGATGTC CTGAACCAAA GAGCTGTGAC ACTCCACCGC CCTGCCCAGAG 480

GTGCCCAGCA CCTGAACTCC TGGGAGGGTA ATAGCCCGGG 520

35

## (2) INFORMATION FOR SEQ ID NO: 116:

- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: other nucleic acid

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

GTTATTACTC GCTGCCCAAC CAGCCATGGC G

31

15 (2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

30 GCAGCAGGAT AGATTGTTGT AGC

23

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 88 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35



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(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

10 CCGGAATTCT TATTAGTTCA GGTCTCCTC AGAGATCAGC TTCTGCTCCT CGAACTCATG 60  
GTGGTGATGG TGGTGGTACA GGTGTTCC 88

(2) INFORMATION FOR SEQ ID NO: 119:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: other nucleic acid

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

CAATCTATCC TGCTGCTGGG ACTTCTAAAG 30

30 (2) INFORMATION FOR SEQ ID NO: 120:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

35

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

GATTGTTGTA GCTCCCGGGC

20

10 (2) INFORMATION FOR SEQ ID NO: 121:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

25 GGAGCTACAA CAATCTATCC TTCTGCTGGG

30

(2) INFORMATION FOR SEQ ID NO: 122:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

- 181 -

ACGGCACCAA GTACACATAT GG

22

(2) INFORMATION FOR SEQ ID NO: 123:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 90 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

ACGAGAATTC GACCGCTCTG CTGCAGCTGC ACCTCGGAAC CGCCACCGCT GCCACCGCCA 60

20 GAACCGCCAC CGTACAGGTG TTCCAGGACG 90

(2) INFORMATION FOR SEQ ID NO: 124:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2154 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

35 ATGTTGGCAC TCTTGGTTCT GGTGACTGTG GCCCTGGCAT CTGCTCATCA TGGTGGTGAG 60

CACTTTGAAG GCGAGAAGGT GTTCCGTGTT AACGTTGAAG ATGAAAATCA CATTAAACATA 120

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	ATCCGCGAGT TGGCCAGCAC GACCCAGATT GACTTCTGGA AGCCAGATTC TGTCACACAA	180
	ATCAAACCTC ACAGTACAGT TGA CTCTCCGT GTTAAAGCAG AAGATACTGT CACTGTGGAG	240
5	AATGTTCTAA AGCAGAATGA ACTACAATAC AAGGTACTGA TAAGCAACCT GAGAAATGTG	300
	GTGGAGGCTC AGTTTGATAG CCGGGTTCGT GCAACAGGAC ACAGTTATGA GAAGTACAAC	360
	AAGTGGGAAA CGATAGAGGC TTGGACTCAA CAAGTCGCCA CTGAGAATCC AGCCCTCATC	420
10	TCTCGCAGTG TTATCGGAAC CACATTTGAG GGACGCGCTA TTTACCTCCT GAAGGTTGGC	480
	AAAGCTGGAC AAAATAAGCC TGCCATTTTC ATGGACTGTG GTTCCATGC CAGAGAGTGG	540
15	ATTCTCCTG CATTCTGCCA GTGGTTTGTA AGAGAGGCTG TTCGTACCTA TGGACGTGAG	600
	ATCCAAGTGA CAGAGCTTCT CGACAAGTTA GACTTTTATG TCCTGCCTGT GCTCAATATT	660
	GATGGCTACA TCTACACCTG GACCAAGAGC CGATTTTGGA GAAAGACTCG CTCCACCCAT	720
20	ACTGGATCTA GCTGCATTGG CACAGACCCC AACAGAAATT TTGATGCTGG TTGGTGTGAA	780
	ATTGGAGCCT CTCGAAACCC CTGTGATGAA ACTTACTGTG GACCTGCCGC AGAGTCTGAA	840
25	AAGGAGACCA AGGCCCTGGC TGATTTTCATC CGCAACAAAC TCTCTTCCAT CAAGGCATAT	900
	CTGACAATCC ACTCGTACTC CCAAATGATG ATCTACCCTT ACTCATATGC TTACAAACTC	960
	GGTGAGAACA ATGCTGAGTT GAATGCCCTG GCTAAAGCTA CTGTGAAAGA ACTTGCCTCA	1020
30	CTGCACGGCA CCAAGTACAC ATATGGCCCG GGAGCTACAA CAATCTATCC TTCTGCTGGG	1080
	ACTTCTAAAG ACTGGGCTTA TGACCAAGGA ATCAGATATT CCTTCACCTT TGAACCTCGA	1140
35	GATACAGGCA GATATGGCTT TCTCCTTCCA GAATCCCAGA TCCGGGCTAC CTGCGAGGAG	1200
	ACCTTCCTGG CAATCAAGTA TGTTGCCAGC TACGTCTGG AACACCTGTA CGGTGGCGGT	1260

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TCTGGCGGTG GCAGCGGTGG CGGTTCCGAG GTGCAGCTGC AGCAGAGCGG TCCAGGTCTC 1320

GTACGGCCTA GCCAGACCCT GAGCCTCAGC TGCACCGCAT CTGGCTTCAA CATTAAAGGAC 1380

5 AATTACATGC ACTGGGTGAG ACAGCCACCT GGACGAGGCC TTGAGTGGAT TGGATGGATT 1440

GACCCTGAGA ATGGTGACAC TGAGTACGCA CCTAAGTTTC GCGGCCGCGT GACAATGCTG 1500

GCAGACACTA GTAAGAACCA GTTCAGCCTG AGACTCAGCA GCGTGACAGC CGCCGACACC 1560

10 GCGGTCTATT ATTGTCACGT CCTGATATAC GCCGGGTATC TGGCAATGGA CTACTGGGGC 1620

CAAGGGACCC TCGTCACCGT GAGCTCGGCT AGCACCAAGG GCCCATCGGT CTTCCCCCTG 1680

15 GCGCCCTGCT CCAGGAGCAC CTCTGGGGGC ACAGCGGCCC TGGGCTGCCT GGTCAAGGAC 1740

TACTTCCCCG AACCGGTGAC GGTGTCGTGG AACTCAGGCG CCCTGACCAG CGGCGTGAC 1800

ACCTTCCCGG CTGTCCTACA GTCTCAGGA CTCTACTCCC TCAGCAGCGT GGTGACCGTG 1860

20 CCCTCCAGCA GCTTGGGCAC CCAGACCTAC ACCTGCAACG TGAATCACA GCCCAGCAAC 1920

ACCAAGGTGG ACAAGAGAGT GGAGCTGAAA ACCCCACTCG GTGACACAAC TCACACGTGC 1980

25 CCTAGGTGTC CTGAACCTAA ATCTGTGAC ACACCTCCCC CGTGCCCAGG GTGCCCAGAG 2040

CCCAAATCTT GCGACAGCC CCCACCGTGT CCCAGATGTC CTGAACCAA GAGCTGTGAC 2100

ACTCCACCGC CCTGCCCAGG GTGCCCAGCA CCTGAACTCC TGGGAGGGTA ATAG 2154

30

(2) INFORMATION FOR SEQ ID NO: 125:

## (i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 716 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

10

Met Leu Ala Leu Leu Val Leu Val Thr Val Ala Leu Ala Ser Ala His  
1 5 10 15

His Gly Gly Glu His Phe Glu Gly Glu Lys Val Phe Arg Val Asn Val  
20 25 30

15

Glu Asp Glu Asn His Ile Asn Ile Ile Arg Glu Leu Ala Ser Thr Thr  
35 40 45

Gln Ile Asp Phe Trp Lys Pro Asp Ser Val Thr Gln Ile Lys Pro His  
50 55 60

20

Ser Thr Val Asp Phe Arg Val Lys Ala Glu Asp Thr Val Thr Val Glu  
65 70 75 80

Asn Val Leu Lys Gln Asn Glu Leu Gln Tyr Lys Val Leu Ile Ser Asn  
85 90 95

25

Leu Arg Asn Val Val Glu Ala Gln Phe Asp Ser Arg Val Arg Ala Thr  
100 105 110

30

Gly His Ser Tyr Glu Lys Tyr Asn Lys Trp Glu Thr Ile Glu Ala Trp  
115 120 125

35

Thr Gln Gln Val Ala Thr Glu Asn Pro Ala Leu Ile Ser Arg Ser Val  
130 135 140

Ile Gly Thr Thr Phe Glu Gly Arg Ala Ile Tyr Leu Leu Lys Val Gly  
145 150 155 160

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	Lys	Ala	Gly	Gln	Asn	Lys	Pro	Ala	Ile	Phe	Met	Asp	Cys	Gly	Phe	His	
						165					170					175	
5	Ala	Arg	Glu	Trp	Ile	Ser	Pro	Ala	Phe	Cys	Gln	Trp	Phe	Val	Arg	Glu	
						180					185					190	
	Ala	Val	Arg	Thr	Tyr	Gly	Arg	Glu	Ile	Gln	Val	Thr	Glu	Leu	Leu	Asp	
						195					200					205	
10	Lys	Leu	Asp	Phe	Tyr	Val	Leu	Pro	Val	Leu	Asn	Ile	Asp	Gly	Tyr	Ile	
						210					215					220	
	Tyr	Thr	Trp	Thr	Lys	Ser	Arg	Phe	Trp	Arg	Lys	Thr	Arg	Ser	Thr	His	
						225					230					235	
15																	
	Thr	Gly	Ser	Ser	Cys	Ile	Gly	Thr	Asp	Pro	Asn	Arg	Asn	Phe	Asp	Ala	
						245						250				255	
	Gly	Trp	Cys	Glu	Ile	Gly	Ala	Ser	Arg	Asn	Pro	Cys	Asp	Glu	Thr	Tyr	
20						260					265					270	
	Cys	Gly	Pro	Ala	Ala	Glu	Ser	Glu	Lys	Glu	Thr	Lys	Ala	Leu	Ala	Asp	
						275					280					285	
25	Phe	Ile	Arg	Asn	Lys	Leu	Ser	Ser	Ile	Lys	Ala	Tyr	Leu	Thr	Ile	His	
						290					295					300	
	Ser	Tyr	Ser	Gln	Met	Met	Ile	Tyr	Pro	Tyr	Ser	Tyr	Ala	Tyr	Lys	Leu	
						305					310					315	
30																	
	Gly	Glu	Asn	Asn	Ala	Glu	Leu	Asn	Ala	Leu	Ala	Lys	Ala	Thr	Val	Lys	
						325					330					335	
	Glu	Leu	Ala	Ser	Leu	His	Gly	Thr	Lys	Tyr	Thr	Tyr	Gly	Pro	Gly	Ala	
35						340					345					350	
	Thr	Thr	Ile	Tyr	Pro	Ser	Ala	Gly	Thr	Ser	Lys	Asp	Trp	Ala	Tyr	Asp	
						355					360					365	

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Gln Gly Ile Arg Tyr Ser Phe Thr Phe Glu Leu Arg Asp Thr Gly Arg  
 370 375 380

5 Tyr Gly Phe Leu Leu Pro Glu Ser Gln Ile Arg Ala Thr Cys Glu Glu  
 385 390 395 400

Thr Phe Leu Ala Ile Lys Tyr Val Ala Ser Tyr Val Leu Glu His Leu  
 405 410 415

10

Tyr Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Val Gln  
 420 425 430

15

Leu Gln Gln Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser  
 435 440 445

Leu Thr Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asn Tyr Met His  
 450 455 460

20

Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly Trp Ile  
 465 470 475 480

Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe Arg Gly Arg  
 485 490 495

25

Val Thr Met Leu Ala Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg Leu  
 500 505 510

30

Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys His Val Leu  
 515 520 525

35

Ile Tyr Ala Gly Tyr Leu Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu  
 530 535 540

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu  
 545 550 555 560



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Ala Pro Cys Ser Arg Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys  
565 570 575

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser  
5 580 585 590

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser  
595 600 605

10 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser  
610 615 620

Leu Gly Thr Gln Thr Tyr Thr Cys Asn Val Asn His Lys Pro Ser Asn  
625                      630                      635                      640

15

Thr Lys Val Asp Lys Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr

645 650 655

Thr His Thr Cys Pro Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro  
20                   660                   665                   670

Pro Pro Cys Pro Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro  
675 680 685

25      Pro Cys Pro Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro  
            690                    695                    700

Cys Pro Arg Cys Pro Ala Pro Glu Leu Leu Gly Gly  
705 710 715

30

(2) INFORMATION FOR SEQ ID NO: 126:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

35

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

TATATAAAGC TTGCCGCCAC CATGGGCCAC ACACGGAGGC AG

42

10 (2) INFORMATION FOR SEQ ID NO: 127:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

25 ACTCCACCAG CTCACCTCG TTATCAGGAA AATGCTCTTG CTTGG

45

(2) INFORMATION FOR SEQ ID NO: 128:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

AGAGCATTTT CCTGATAACG AGGTGAAGCT GGTGGAGTCT GGAGG

45

5

(2) INFORMATION FOR SEQ ID NO: 129:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

10

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:

20

CCAGGCATCC CAGGGTCACC ATGGAGTTAG TTTGGGCAGC

40

(2) INFORMATION FOR SEQ ID NO: 130:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1446 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: other nucleic acid

(ix) FEATURE:

35

(A) NAME/KEY: CDS

(B) LOCATION: 16..1435

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

	AAGCTTGCCG CCACC ATG GGC CAC ACA CGG AGG CAG GGA ACA TCA CCA TCC	51
	Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser	
5	1 5 10	
	AAG TGT CCA TAC CTC AAT TTC TTT CAG CTC TTG GTG CTG GCT GGT CTT	99
	Lys Cys Pro Tyr Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu	
	15 20 25	
10		
	TCT CAC TTC TGT TCA GGT GTT ATC CAC GTG ACC AAG GAA GTG AAA GAA	147
	Ser His Phe Cys Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu	
	30 35 40	
15	GTG GCA ACG CTG TCC TGT GGT CAC AAT GTT TCT GTT GAA GAG CTG GCA	195
	Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala	
	45 50 55 60	
	CAA ACT CGC ATC TAC TGG CAA AAG GAG AAG AAA ATG GTG CTG ACT ATG	243
20	Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met	
	65 70 75	
	ATG TCT GGG GAC ATG AAT ATA TGG CCC GAG TAC AAG AAC CGG ACC ATC	291
	Met Ser Gly Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile	
25	80 85 90	
	TTT GAT ATC ACT AAT AAC CTC TCC ATT GTG ATC CTG GCT CTG CGC CCA	339
	Phe Asp Ile Thr Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro	
	95 100 105	
30		
	TCT GAC GAG GGC ACA TAC GAG TGT GTT GTT CTG AAG TAT GAA AAA GAC	387
	Ser Asp Glu Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp	
	110 115 120	
35	GCT TTC AAG CGG GAA CAC CTG GCT GAA GTG ACG TTA TCA GTC AAA GCT	435
	Ala Phe Lys Arg Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala	
	125 130 135 140	

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	GAC TTC CCT ACA CCT AGT ATA TCT GAC TTT GAA ATT CCA ACT TCT AAT	483
	Asp Phe Pro Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn	
	145 150 155	
5	ATT AGA AGG ATA ATT TGC TCA ACC TCT GGA GGT TTT CCA GAG CCT CAC	531
	Ile Arg Arg Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His	
	160 165 170	
	CTC TCC TGG TTG GAA AAT GGA GAA GAA TTA AAT GCC ATC AAC ACA ACA	579
10	Leu Ser Trp Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr	
	175 180 185	
	GTT TCC CAA GAT CCT GAA ACT GAG CTC TAT GCT GTT AGC AGC AAA CTG	627
	Val Ser Gln Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu	
15	190 195 200	
	GAT TTC AAT ATG ACA ACC AAC CAC AGC TTC ATG TGT CTC ATC AAG TAT	675
	Asp Phe Asn Met Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr	
	205 210 215 220	
20		
	GGA CAT TTA AGA GTG AAT CAG ACC TTC AAC TGG AAT ACA ACC AAG CAA	723
	Gly His Leu Arg Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln	
	225 230 235	
25	GAG CAT TTT CCT GAT AAC GAG GTG AAG CTG GTG GAG TCT GGA GGA GGC	771
	Glu His Phe Pro Asp Asn Glu Val Lys Leu Val Glu Ser Gly Gly Gly	
	240 245 250	
30	TTG GTA CAG CCT GGG GGT TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG	819
	Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly	
	255 260 265	
	TTC ACC TTC ACT GAT TAC TAC ATG AAC TGG GTC CGC CAG CCT CCA GGA	867
35	Phe Thr Phe Thr Asp Tyr Tyr Met Asn Trp Val Arg Gln Pro Pro Gly	
	270 275 280	
	AAG GCA CTT GAG TGG TTG GGT TTT ATT GGA AAC AAA GCT AAT GGT TAC	915

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Lys Ala Leu Glu Trp Leu Gly Phe Ile Gly Asn Lys Ala Asn Gly Tyr  
285                      290                      295                      300

ACA ACA GAG TAC AGT GCA TCT GTG AAG GGT CGG TTC ACC ATC TCC AGA 963  
5 Thr Thr Glu Tyr Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg  
305 310 315

GAC AAA TCC CAA AGC ATC CTC TAT CTT CAA ATG AAC ACC CTG AGA GCT 1011  
Asp Lys Ser Gln Ser Ile Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala  
10 320 325 330

GAG GAC AGT GCC ACT TAT TAC TGT ACA AGA GAT AGG GGG CTA CGG TTC 1059  
Glu Asp Ser Ala Thr Tyr Tyr Cys Thr Arg Asp Arg Gly Leu Arg Phe  
335 340 345

15

TAC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC 1107

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala

350 355 360

20 AAA ACG ACA CCC CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC 1155  
Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala  
365 370 375 380

CAA ACT AAC TCC ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC 1203  
25 Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe  
385 390 395

CCT GAG CCA GTG ACA GTG ACC TGG AAC TCT GGA TCT CTG TCC AGC GGT 1251  
Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly

GTG CAC ACC TTC CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC 1299  
Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser  
415 420 425

35  
AGC TCA GTG ACT GTC CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC 1347  
Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr  
430 435 440

- 193 -

TGC AAC GTT GCC CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT 1395  
 Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile  
 445 450 455 460

5

GTG CCC AGG GAT TGT GGT TGT AAG CCT TGC ATA TGT ACA T AGTAAGAATT 1445  
 Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr  
 465 470

10 c

1446

(2) INFORMATION FOR SEQ ID NO: 131:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 473 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr

1

5

10

15

25

Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys

20

25

30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu

30

35

40

45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile

50

55

60

35 Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp

65

70

75

80

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Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr  
                     85                    90                    95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly  
 5                    100                    105                    110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg  
                     115                    120                    125

10 Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr  
                     130                    135                    140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile  
                     145                    150                    155                    160

15 Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu  
                     165                    170                    175

20 Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp  
                     180                    185                    190

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met  
                     195                    200                    205

25 Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg  
                     210                    215                    220

Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro  
 30 225                    230                    235                    240

Asp Asn Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro  
                     245                    250                    255

35 Gly Gly Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Thr  
                     260                    265                    270



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Asp Tyr Tyr Met Asn Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 275 280 285

Trp Leu Gly Phe Ile Gly Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr  
 5 290 295 300

Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ser Gln  
 305 310 315 320

10 Ser Ile Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala  
 325 330 335

Thr Tyr Tyr Cys Thr Arg Asp Arg Gly Leu Arg Phe Tyr Phe Asp Tyr  
 340 345 350

15 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro  
 355 360 365

Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser  
 20 370 375 380

Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val  
 385 390 395 400

25 Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe  
 405 410 415

Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr  
 420 425 430

30 Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala  
 435 440 445

His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp  
 35 450 455 460

Cys Gly Cys Lys Pro Cys Ile Cys Thr  
 465 470

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**CLAIMS**

1. An anti-CEA antibody ("806.077 Ab") comprising complementarity determining regions (CDRs) in which the CDRs comprise the following sequences:

5 a) heavy chain

CDR1 DNYMH (SEQ ID NO: 29)

CDR2 WIDPENGDT EYAPKFRG (SEQ ID NO: 31)

CDR3 LIYAGYLAMD Y (SEQ ID NO: 32); and

b) light chain

10 CDR1 SASSSVTYMH (SEQ ID NO: 26)

CDR2 STSNLAS (SEQ ID NO: 27)

CDR3 QQRSTYPLT (SEQ ID NO: 28).

2. An antibody according to claim 1 in which the heavy chain CDRs 1 and 3 are further

15 defined as:

CDR1 FNIKDNMYMH (SEQ ID NO: 30); and

CDR3 HVLIYAGYLA MDY (SEQ ID NO: 33).

3. An antibody according to claim 1 comprising the following, optionally humanised,

20 structure:

a heavy chain variable region sequence (SEQ ID NO: 11)

EVQLQQSGAE LVRSGASVKL SCTASGFNIK DNYMHVVKQR	40
PEQGLEWIAW IDPENGDT EYAPKFRGKATL TADSSSNTAY	80
LHLSSLTSED TAVYYCHVLI YAGYLAMDYW GQGTSAVSS	120

25 and;

a light chain variable region sequence (SEQ ID NO: 9):

DIELTQSPAI MSASPGEKVT ITCSASSSVT YMHWFQKPG	40
TSPKLWIYST SNLASGVPAR FSGSGSGTSY SLTISRMEAE	80
DAATYYCQQR STYPLTFGAG TKLELKRA	108.

30

4. A humanised antibody according to claim 3 comprising at least one of the following sequences:

a heavy chain variable region sequence which is VH1 (SEQ ID NO: 55);

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- a light chain variable region sequence which is VK4 (SEQ ID NO: 71);  
 a human CH1 heavy chain IgG3 constant region;  
 a human kappa light chain CL region; and  
 a human IgG3 hinge region;
- 5 optionally in the form of a F(ab')<sub>2</sub> fragment.
5. A conjugate comprising an antibody according to any preceding claim and an effector moiety.
- 10 6. A conjugate according to claim 5 in which the effector moiety is selected from any one of the following:
- a) an enzyme suitable for use in an ADEPT system;
  - b) CPG2;
  - c) [G251T,D253K]HCPB;
  - 15 d) [A248S,G251T,D253K]HCPB;
  - e) a co-stimulatory molecule;
  - f) extracellular domain of B7;
  - g) extracellular domain of human B7.1; and
  - h) extracellular domain of human B7.2;
- 20 optionally in the form of a fusion protein.
7. A conjugate according to claim 6 which is a fusion protein selected from any one of the following conjugates, (sequences being listed in N terminus to C terminus direction):
- a) a humanised 806.077 F(ab')<sub>2</sub> - {[A248S,G251T,D253K]HCPB}<sub>2</sub> fusion comprising:
- 25 an antibody Fd' chain of structure VH1(SEQ ID NO: 55)/CH1 constant region from IgG3/hinge region from IgG3;  
 the Fd' chain being fused via its C terminus to the N terminus of [A248S,G251T,D253K]HCPB; and  
 an antibody light chain of formula VK4(SEQ ID NO: 71)/CL region from kappa light chain;
- 30 b) {[A248S,G251T,D253K]HCPB}<sub>2</sub>-humanised 806.077 F(ab')<sub>2</sub> fusion comprising:  
 [A248S,G251T,D253K]HCPB;

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the HCPB being fused at its C terminus, via a (GGGS)<sub>3</sub> linker, to the N terminus of an antibody Fd' chain of structure VH1(SEQ ID NO: 55)/CH1 constant region from IgG3/hinge region from IgG3; and

an antibody light chain of formula VK4(SEQ ID NO: 71)/CL region from kappa light chain;

5 and

c) a (human B7.1 extracellular domain)<sub>2</sub> - humanised 806.077 F(ab')<sub>2</sub> fusion comprising:

human B7.1 extracellular domain;

the B7.1 being fused at its C terminus to the N terminus of an antibody Fd' chain of structure

10 VH1(SEQ ID NO: 55)/CH1 constant region from IgG3/hinge region from IgG3; and

an antibody light chain of structure VK4(SEQ ID NO: 71)/CL region from kappa light chain.

8. A polynucleotide sequence capable of encoding a polypeptide of an antibody or a conjugate as defined in any preceding claim.

15

9. A vector comprising a polynucleotide as defined in claim 8.

10. A host cell transformed with a polynucleotide sequence as defined in claim 8 or a transgenic non-human animal or transgenic plant developed from the host cell.

20

11. Hybridoma 806.077 deposited as ECACC deposit no. 96022936.

12. A pharmaceutical composition comprising a conjugate as defined in any preceding claim in association with a pharmaceutically-acceptable diluent or carrier, optionally in a form

25 suitable for intravenous administration.

13. A conjugate as described in any preceding claim for use as a medicament.

14. A method of making an antibody or a conjugate as defined in any preceding claim

30 which comprises:

a) subjecting a host cell, a transgenic non-human mammal or a transgenic plant as defined in claim 10, or the hybridoma of claim 11, to conditions conducive to expression, and

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optionally secretion, of the antibody or conjugate; and optionally

b) at least partially purifying the antibody or conjugate.

15. A method of treatment of a human or animal in need of such treatment which  
5 comprises administration to a human or animal of a pharmaceutically effective amount of a  
conjugate as defined in any preceding claim.

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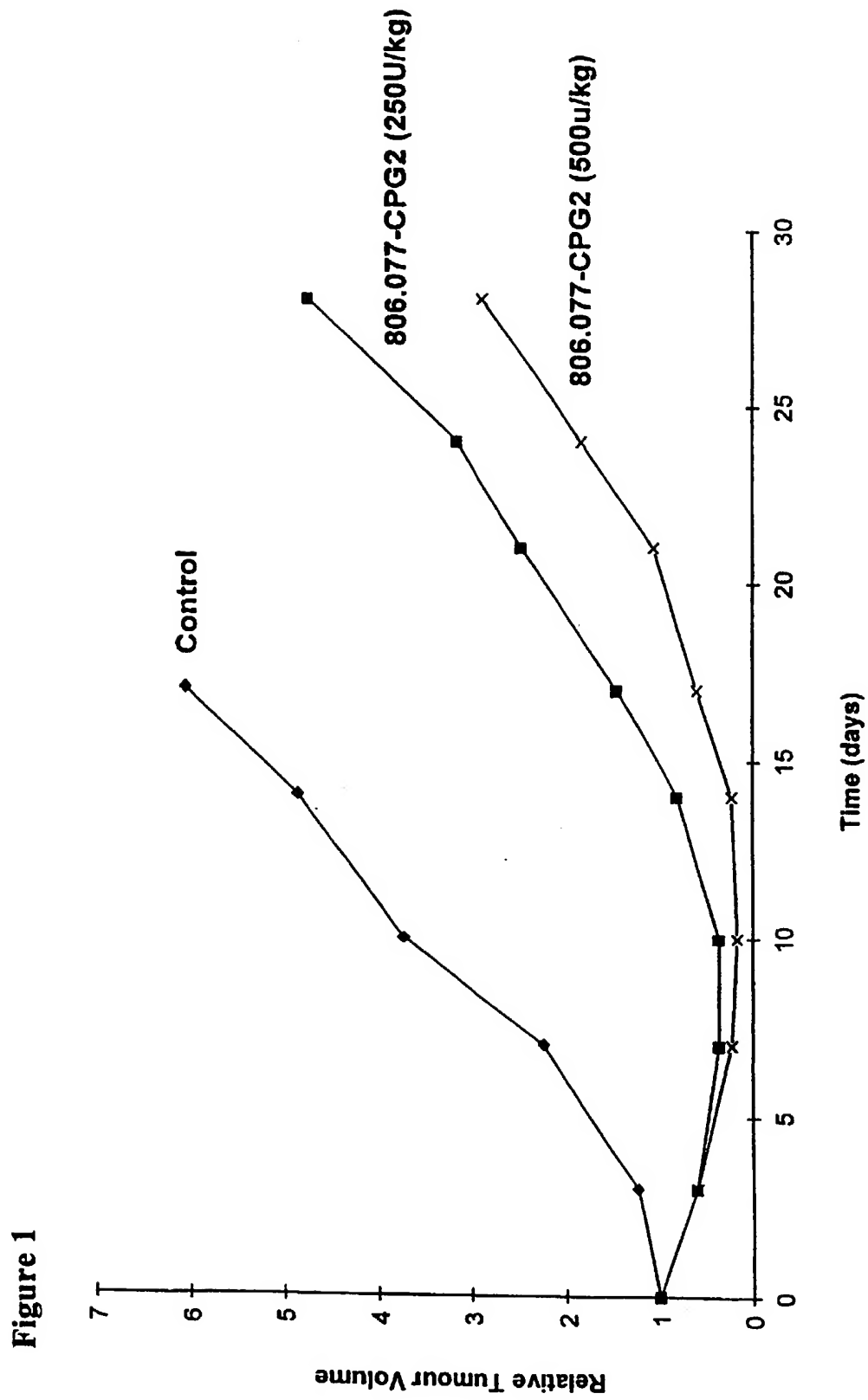
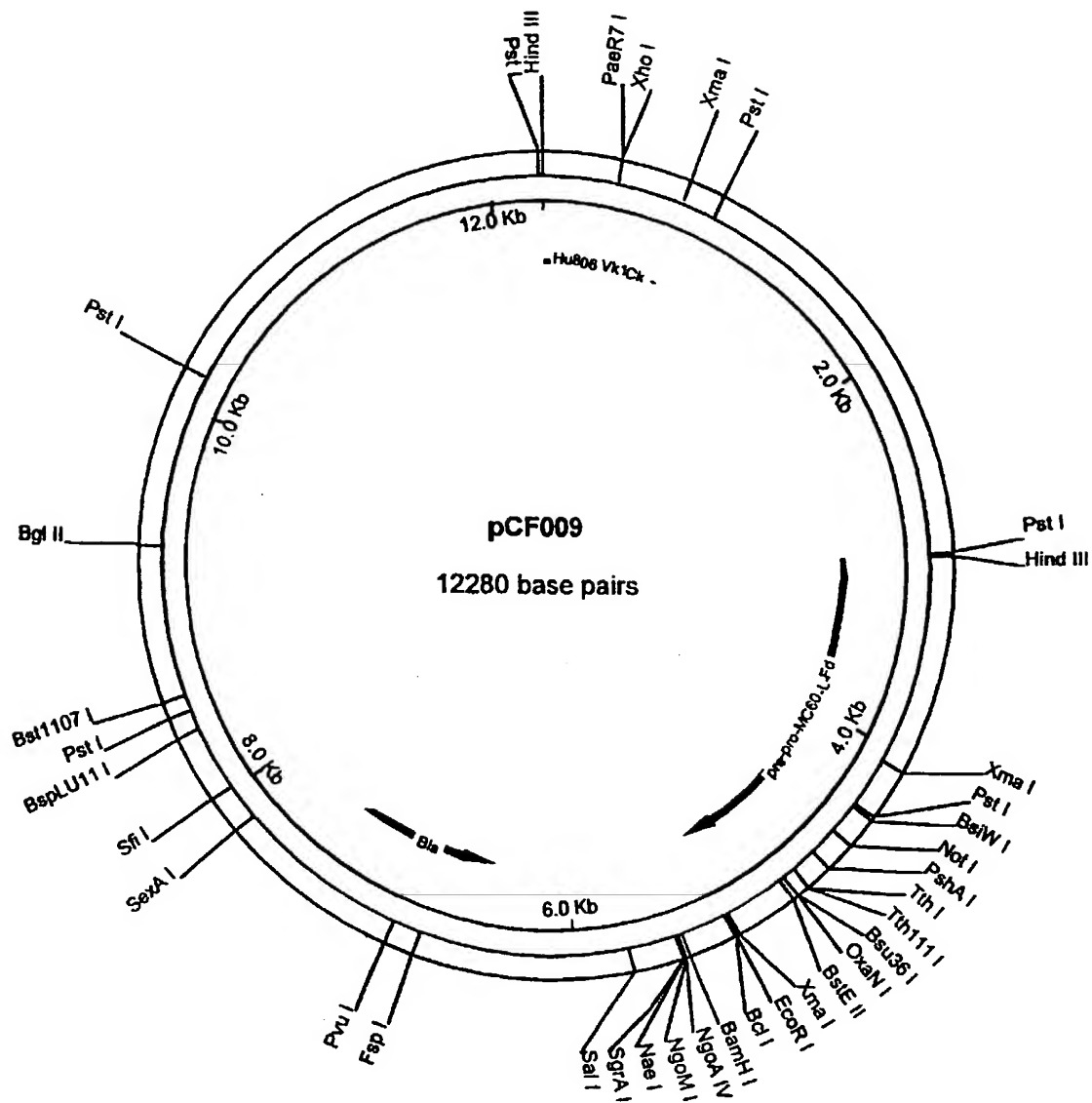


Figure 2

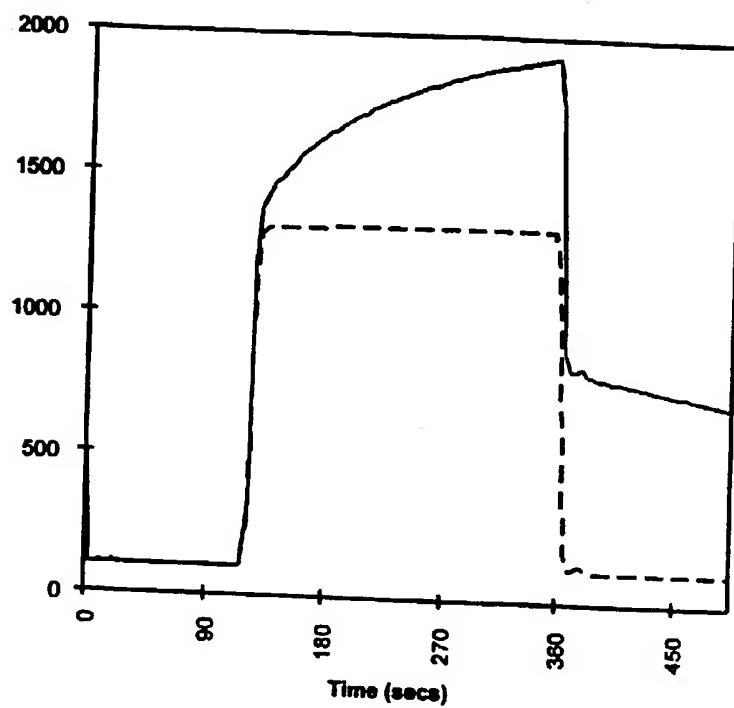


Single cut restriction enzymes

- + HindIII cuts twice
- + XmaI cuts three times
- + PstI cuts six times.

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FIGURE 3





# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/01165

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/62 C12N15/13 C07K16/30 C07K16/46 A61K47/48  
C07K19/00 C07K14/705 C12N9/64 C12N5/10 C12N1/21  
C12N5/20 //A01K67/027, A01H1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 633 029 A (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 11 January 1995 see claims	1-6, 12-15
X	BRITISH JOURNAL OF CANCER, vol. 72, no. 5, November 1995, BASINGSTOKE, GB, pages 1083-1088, XP002038652 D. BLAKEY ET AL.: "Anti-tumour effects of an antibody-carboxypeptidase G2 conjugate in combination with phenol mustard prodrugs." see the whole document	1-3,5,6, 12-15

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \* "A" document defining the general state of the art which is not considered to be of particular relevance
- \* "E" earlier document but published on or after the international filing date
- \* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \* "O" document referring to an oral disclosure, use, exhibition or other means
- \* "P" document published prior to the international filing date but later than the priority date claimed

- \* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \* "A" document member of the same patent family

Date of the actual completion of the international search

22 August 1997

Date of mailing of the international search report

04.09.97

Name and mailing address of the ISA

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Nooij, F

## INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/GB 97/01165

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 15341 A (CANCER RESEARCH CAMPAIGN TECHNOLOGY LTD.) 8 June 1995  see example 4 see figure 3 see seq. id. nos 1, 2 ---	1-3, 5-10, 12-15
X	IMMUNOTECHNOLOGY, vol. 2, no. 1, 1996, AMSTERDAM, NL, pages 47-57, XP002038653 N. MICHAEL ET AL.: "In vitro and in vivo characterization of a recombinant carboxypeptidase G2::anti-CEA scFv fusion protein." see the whole document ---	1-3, 5-10, 12-15
X	WO 96 11013 A (IMMUNOMEDICS) 18 April 1996  see examples see claims ---	1-5, 8-10, 12-15
X	CANCER RESEARCH, vol. 55, no. 23 suppl., 1 December 1995, BALTIMORE, MD, USA, pages 5968S-5972S, XP002038654 S. LEUNG ET AL.: "Bacterial expression of a kemptide fusion protein facilitates 32P labeling of a humanized, anti-carcinoembryonic antigen (hMN-14) antibody fragment." see abstract ---	1-5, 8-10, 12-15
X	WO 92 01059 A (CELLTECH LTD.) 23 January 1992 see examples see claims ---	1-4,8-10
X	CANCER RESEARCH, vol. 54, no. 7, 1 April 1994, BALTIMORE, MD, USA, pages 2151-2159, XP002038655 K. BOSSLET ET AL.: "Tumor-selective prodrug activation by fusion protein-mediated catalysis." see abstract ---	1-6, 12-15
A	WO 94 25585 A (GENPHARM INTERNATIONAL, INC.) 10 November 1994 see example 22 see claims -----	8-10,14

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 97/ 01165

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 15  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 15 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 97/01165

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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